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(57) Abstract

Polypeptides capable of forming antigen binding structures specific for Rhesus D antigens include the sequences indicated in the figures 1a to 16b. The obtained polypeptides, being Fab fragments, may be used directly as an active ingredient in pharmaceutical and diagnostic compositions. The Fab and their DNA sequences can also be used for the preparation of complete recombinant Anti-Rhesus D antibodies. Useful in pharmaceutical and diagnostic compositions.

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Polypeptides capabl of forming antigen binding structur s with specificity for the Rhesus D antigens, the DNA incoding them and the process for their preparation and use

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This invention relates to polypeptides forming antigen binding structures with specificity for Rhesus D antigens and especially to Fab molecules with specificity for the Rhesus D antigen. The invention also relates to their application to provide pharmacological and diagnostic compositions. The above Fab fragments when genetically engineered to be part of complete antibodies are useful for the prophylaxis of hemolytic disease of the newborn (HDN). This invention provides the novel DNA and amino acid sequences of the above polypeptides.

Thus, the antibodies can be used for the protection of Rhesus negative women before or immediately after the birth of a Rhesus positive child to prevent HDN in subsequent pregnancies.

The invention also includes the application of the said Fab molecules either alone or in combination with Fc constant regions as complete antibodies for the purposes of treating other illnesses which might benefit from anti-Rhesus D immunoglobulin e.g. treatment of idiopathic thrombocytopenic purpura (ITP).

In addition anti-Rhesus D immunoglobulin can be used after mistransfusions of Rhesus positive blood to Rhesus negative recipients in order to prevent sensitization to the Rhesus D antigen. Further the invention relates to the application of these Fab fragments and antibodies as diagnostic reagents.

HDN is the general designation for hemolytic anemia of fetuses and newborn babies caused by antibodies of the mother. These antibodies are directed against antigens on the surface of the fetal erythrocytes. These antigens can belong to the Rhesus, ABO or other blood group systems.

The Rhesus blood group system includes 5 major antigens: D, C, c, E and e (Issitt, P.D., Med. Lab. Sci. 45:395, 1988). The D antigen is the most important of these antigens as it is highly immunogenic eliciting anti-Rhesus D antibodies during Rhesus incompatible pregnancies and following transfusion of Rhesus incompatible blood. The D antigen is found in approximately 85% of Caucasians in Europe and those individuals are said to

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be Rhesus positive. Individuals lacking the D antigen are called Rhesus negative. The expression of the D antigen can vary due to either low antigen density, hereafter known as weak D or D^u, or due to partial antigenicity, hereafter known as partial D antigens.

The Rhesus D antigen, a membrane protein of the erythrocyte, has recently been cloned and its primary structure described (Le Van Kim, C., et al., PNAS 89:10925, 1992). Modeling studies suggest that the Rhesus D antigen has 12 transmembrane domains with only very short connecting regions extending outside the cell membrane or protruding into the cytoplasm.

The partial D phenotypes were first identified in people who carried D antigen on their red cells but who had an alloanti-D in their sera (Rose, R. R. and Sanger, R., Blood groups in man, Blackwell Scientific, Oxford, U.K. 1975; Tippett, P. et al., Vox Sanguinis. 70:123, 1996). This can be explained by regarding the D antigen as a mosaic structure with at least 9 different epitopes (epD1 to epD9). Thus in some D variant people the red cells lack part of this mosaic and antibodies are made to the missing D epitopes. Rhesus positive individuals that make antibodies against partial D antigens have been classified into six main different categories (DIII to DVIII) each having a different abnormality in the D antigen. More recently it has been shown that these D categories gave different patterns of reaction when tested against panels of human monoclonal anti-D antibodies (Tippett, P., et al., Vox Sanguinis. 70:123, 1996). The different reaction patterns identified the 9 epitopes and so define the different partial D categories. The number of epitopes present on the D antigen varies from one partial D category to another with the D^{VI} category expressing the least, epD3, 4 and 9. The D^{VI} category is clinically important as a D^{VI} woman can be immunized strongly enough to cause hemolytic disease of the newborn.

The prophylactic efficacy of anti-RhD IgG for prevention of hemolytic disease of the newborn is well established and has been in routine use for many years. As a result this severe disease has become a rarity. Nevertheless the underlying cause of the disease, i.e. RhD incompatibility between a RhD negative mother carrying a RhD positive child still remains and thus requires a continual supply of therapeutic anti-RhD IgG.

In recent years the assurance of a continual supply of anti-RhD lgG has become an increasing problem. The pool of available hyperimmune

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serum from alloimmunized multiparous Rhesus negative women has drastically decreased due to the success of prophylactic anti-RhD. Thus the current methods of production require repeated immunization of an increasingly reluctant pool of donors for the production of high titer antiserum 5 (Selinger, M., Br. J. Obstet. Gynaecol. 98:509, 1991). There are also associated risk factors and technical problems such as the use of Rhesus positive red blood cells for repeated immunization carrying the risk of transmission of viral diseases like hepatitis B, AIDS and other as yet unknown viruses (Hughes-Jones, N.C., Br. J. Haematol. 70:263, 1988). Therefore an alternative method for production of anti-RhD antibodies is required.

In the past few years various alternative sources of hyperimmune serum have been tried but all are associated with disadvantages. Epstein Barr Virus (EBV) transformation of lymphocytes creating B lymphoblastoid cell lines that secrete specific antibody including against the Rhesus D antigen (Crawford et al., Lancet. 386:Feb.19th, 1983) are unstable and require extensive cloning. Also due to the low transformation efficiencies (1-3% of B cells) only a restricted range of antibody specificities can be obtained from the potential repertoire. Additionally it seems that mice do not respond to the Rhesus D antigen and thus no murine monoclonal antibodies are available which could be used for producing chimaeric or humanised antibodies. Until recently the only other alternative was production of human antibodies by the hybridoma technique which was also restricted by the lack of a suitable human myeloma cell fusion partner (Kozbor, D. and Roder, J.C., Immunol. Today, 4:72, 1983).

It is thus the object of the present invention to provide Fab fragments having a reactivity against the Rhesus D antigen as well as complete antibodies comprising the Fab fragments which are free from the above mentioned drawbacks.

In the last few years the technique of repertoire cloning and the construction of phage display libraries has opened up new possibilities to produce human antibodies of defined specificity (Williamson, R.A. et al., PNAS 90:4141, 1993). These methods were thus applied to the preparation of polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens, especially of Fab fragments having an activity against

Rhesus D and partial D antigens. 35

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The generation of human antibodies by repertoire cloning as described in recent years (Barbas III, C.F. and Lerner, R.A., Companion Methods Enzymol. 2:119, 1991) is based on isolating mRNA from peripheral B cells. This method offers the tools to isolate natural antibodies. autoantibodies or antibodies generated during the course of an immune response (Zebedee, S.L., et al., PNAS 89:3175, 1992; Vogel, M. et al., Eur.J. Immunol. 24:1200, 1994). This method relies on constructing a recombinant antibody library from a particular donor starting from the mRNA coding for immunoglobulin (Ig) molecules. As only the peripheral blood lymphocytes (PBL) can be isolated from a donor the chances of finding specific antibody producing B cells in the periphery are increased if an individual is boosted with the desired antigen shortly before harvesting the PBL (Persson, M.A.A., et al., PNAS 88:2432, 1991). The total RNA is then isolated and the mRNA of the Ig repertoire can be cloned using Ig specific primers in the polymerase chain reaction (PCR) followed by the co-expression of heavy and light chains of the Ig molecule on the surface of a filamentous phage particle thereby forming an "organism" that in analogy to a B cell can bind to an antigen. In the literature this method is also known as the combinatorial approach as it allows the independent combining of heavy and light chains to form a functional Fab antibody fragment attached to one of the tail proteins, called pIII, of a filamentous phage. Phages carrying the Fab molecules (hereafter known as Phab particles) are selected for the desired antigen specificity, by a process known as bio-panning. The antigen can be applied to a solid support, specific Phab bind to the antigen whilst non specific Phab are washed away and finally the specific Phab are eluted from the solid support. The specific Phab are then amplified in bacteria, allowed to re-bind to the antigen on the solid support and the whole process of bio-panning is repeated.

The successive rounds of panning and amplification of selected Phab in bacteria result in an enrichment of specific Phab that can be seen from a rise in titer of colony forming units (cfu) plated out after each round of panning. Our previous experience and published data indicate that specific phage can usually be detected after 4 to 6 panning rounds (Vogel, M. et al., Eur.J. Immunol. 24:1200, 1994). In the above cited related art there is, however, no hint that the indicated steps can be used for a successful preparation of Fab fragments of anti-Rh D antibodies.

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In the appended figures 1a to 16b; DNA sequences coding for variable regions (V regions) of anti Rh D Fab fragments and the corresponding polypeptide sequences are disclosed.

Fig. 17 shows the pComb3 expression system used according to the present invention.

Figs. 18 and 19 show the separate preparation of genes of the heavy and light chains of the complete antibody according to the description in example 6.

Subjects of the present invention are polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens according to the definition of claim 1. The table in claim 1 refers to the appended figures. The identification number for each sequence is given. The locations of the Rhesus D specific CDR1 (complementarity determining region 1), CDR2 and CDR3 regions are indicated in the figures and according to base pair number in the table of claim 1. Preferred polypeptides according to the invention are anti-Rhesus D antibodies which include the variable regions of the heavy and light chains according to the sequences given in Figs. 1a -16b. The Figs. 1a, 2a, ... 16a are related to the variable regions of the light chain.

Further subjects of the present invention are the DNA sequences coding for antigen binding polypeptides according to the definition of claim 6. Prefered DNA sequences are those coding for variable regions of Fab fragments of anti-Rh D antibodies according to the Figs. 1a -16b. The Figs. 1a, 2a, ... 16a are related to the heavy chain and the Figs. 1b, 2b, ... 16b are related to the light chain.

A further subject of the present invention is a process for preparing recombinant Fab polypeptides according to the definition in claim 11.

A further subject of the present invention is a process for the selection of recombinant polypeptides according to claim 12.

Further subjects of the present invention are anti-Rh D antibodies according to the definition of claim 14, preferably anti-Rh D immunoglobulin molecules comprising the heavy and light chain variable regions according to

the Figs. 1a to 16b combined with known heavy and light chain constant regions.

Further subjects of the present invention are pharmaceutical and diagnostic compositions comprising polypeptides, anti-Rh D antibodies or Fab fragments according to the invention.

The total re-amplified Phab population obtained after each panning can be tested for specificity using various methods such as ELISA and immunodot assays. It is also defined by the nature of the antigen e.g. anti-Rhesus D Phabs are detected by indirect haemagglutination using a rabbit anti-phage antibody or equivalent Coombs reagent as the cross linking antibody. Once a total Phab population has been identified as positive for the desired antigen, individual Phab clones are isolated and the DNA coding for the desired Fab molecules is sequenced. Individual Fab can then be produced by use of the pComb3 expression system which is illustrated in Fig. 16. In this system the gIII gene, coding for the tail protein pIII, is cut out from the phagemid vector pComb3. This allows production of soluble Fab in the bacterial periplasm. Such individual Fab fragments can then be tested for antigen specificity.

The phage display approach has also been used as a means of rescuing monoclonal antibodies from unstable hybridoma cell lines. This has been reported for anti-Rhesus D antibodies (Siegel, D.L. and Silberstein, L.E., Blood. 83:2334, 1994; Dziegiel, M. et al., J. Immunol. Methods. 182:7, 1995). A phage display library constructed from non-immunized donors has also been used to select Fv fragments (i.e. variable regions of heavy and light chains, V_H and V_L) specific for human blood group antigens which included one Fv fragment reacting against the Rhesus D antigen (Marks, J.D. et al., Biotechnology. 11:1145, 1993).

Important considerations when constructing combinatorial libraries are the source of cells used for RNA extraction and the nature of the antigen used for panning. Therefore, this invention uses a hyperimmune donor who was boosted i.v. with Rhesus D⁺ red blood cells (rbc). The PBL of the donor were harvested at +5 and +18 days after the i.v. boost and were used to construct 2 combinatorial libraries hereafter known as library D1 (LD1) and library D2 (LD2) respectively. Double immunofluorescence analysis of the harvested PBL, using the markers CD20 and CD38 for pan B cells and

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lymphoblastoid cells respectively, showed a higher than normal percentage of lymphoblastoid B cells, of plasma cell morphology. The high number of plasma cells found in the peripheral blood is most unusual as normally there are less than 1% in the periphery and probably indicates that the donor had a high percentage of circulating B cells with specificity for the Rhesus D antigen.

After construction of the library, the selection of Phabs specific for the Rhesus D antigen was achieved by bio-panning on fresh whole rbc of phenotype R1R1 (CDe/CDe) i.e. the reference cells used for Rhesus D typing. This was necessary since the Rhesus D antigen, an integral membrane protein of 417 amino acids (Le Van Kim, C. et al, PNAS 89:10925, 1992), loses its immunogenicity during purification (Paradis, G. et al, J. Immunol. 137:240, 1986) and therefore a chemically purified D antigen cannot be bound to a solid phase for selection of immunoreactive Phabs as for other antigen specificities previously selected in this system (Vogel, M. et al., Eur.J. Immunol. 24:1200, 1994). Modelling studies have suggested that only very short connecting regions of the Rhesus D antigen extend outside the cell membrane or protrude into the cytoplasm (Chérif-Zahar, B. et al, PNAS 87:6243, 1990). Thus the parts of the RhD antigen visible to antibodies are relatively restricted and may be under conformational constraint. This aspect of the Rhesus D antigen becomes even more important when considering selection of Phabs with reactivity against the partial D phenotypes which essentially lack certain defined epitopes of the D membrane protein (Mouro, I. et al, Blood. 83:1129, 1994).

Furthermore, since whole rbc do not only express the D antigen, a series of negative absorptions had to be performed on Rhesus D negative rbc in order to absorb out those Phabs reacting with the other antigenic proteins found on the rbc.

This panning procedure performed on Phabs coming from both LD1 and LD2 librairies resulted in the isolation of 6 different Fab producing clones from library LD1, 8 different Fab producing clones from library LD2 and 2 Fab producing clones from the pooled libraries LD1 and LD2.

The nomenclature and the figures where the sequences are listed are given in table 1.

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Table 1

LIBRARY LD1 Clone No.	V _H - Sequence Figure	V _L - Sequence Figure	LIBRARY LD2 Clone No.	V _H - Sequence Figure	V _L - Sequence Figure
LD1-40	1a	1b	LD2-1	6a	6b
LD1-52	2a	2b	LD2-4	7a	7b
LD1-84	3a	3b	LD2-5	8a	. 8b
LD1-110	4a	4b	LD2-10	9a	9b
LD1-117	5a	5b	LD2-11	10a	10b
			LD2-14	11a	11b
			LD2-17	12a	12b
			LD2-20	13a	13b

The above Fab clones show exclusive reactivity against the Rhesus D antigen, 3 of 5 D^u rbc tested and agglutinating reactivity against the Partial D phenotypes as follows: Rh33, DIII, DIVa, DIVb, DVa, DVII,.

However, using the above mentioned R1R1 rbc for panning of the Phabs, no clones were isolated which reacted against the Partial DVI phenotype. As the serum of the original hyperimmune donor tested at the time of construction of the recombinant library, was known to react against the DVI phenotype the recombinant library should also contain the anti-DVI specificity.

In order to select for the DVI reactivity the panning conditions were changed in that different cells were used. A special donor whose rbc had been typed and were known to express the Partial DVI phenotype was used as the source of cells for re-panning the LD1 and LD2 libraries. This second series of pannings was essentially performed in the same way as the first series except for the substitution of DVI rbc for R1R1 rbc and the addition of bromelase treatment to the DVI rbc. The DVI phenotype expresses the least number of Rhesus D epitopes and it is therefore difficult to make antibodies against it. It has been reported that only 15% of unselected polyclonal anti-D and 35% of selected anti-D made by Rhesus D negative subjects reacted with DVI+ cells (Mouro, I. et al, Blood. 83:1129, 1994). Bromelase treatment which removes N- acetylneuraminic acid (sialic acid) from the rbc membrane, was performed in order to render the Rhesus DVI epitopes more accessible during the panning with the pre-absorbed Phabs.

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This second series of pannings on the LD1 library resulted in 1 Fab producing clone LD1-6-17. The nomenclature is given in table 2.

Table 2

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LIBRARY LD1	V _H -Sequence figure	V _∟ -Sequence figure	
Clone No: LD1-6-17	14a	14b	

However this clone was reacting with Rhesus alleles C and E and showing a false positive reaction with DVI positive rbc. This was also due to the phenotype of the DVI donor (Cc DVI ee) who expressed the C allele which was not absorbed out by the Rhesus negative rbc (ccddee).

Thus a third series of pannings on a pool of the LD1 and LD2 libraries was performed using different rbc for the absorption phase. After 6 rounds of panning using both bromelase treated and non treated rbc for both the absorption steps and the elution from DVI positive rbc a total population of Phabs was obtained which reacted exclusively with rbc of phenotype R1R1 (CCDDee) and 2 different donors expressing the DVI variant.

This third series of pannings on the LD1 and LD2 librairies resulted in 2 Fab producing clones reacting with DVI+ rbc. The nomenclature is given in table 3.

Table 3

LIBRARY LD1/LD2	V _H -Sequence figure	V _L -Sequence figure	
Clone No: LD1/2-6-3	15a	15b	
Clone No: LD1/2-6-33	16a	16b	

Thus a total of 16 different anti-Rhesus D Fab clones have been isolated. The DNA from these clones has been isolated and sequenced using Fluorescent Cycle Sequencing on an ABI 373A Sequencing System. The nucleotide and corresponding amino acid sequences of the said Fab clones form the basis of this invention.

Sequence analysis has revealed that several clones were isolated bearing the same V_{H} gene segment but different V_{L} gene segments. This is

the case for the two clones LD2-1 and LD2-10, for the two clones LD2-4 and LD2-11, and for the three clones LD2-14, LD1/2-6-3 and LD1/2-6-33, respectively.

The DNA sequences obtained and Fab fragments are useful for the preparation of complete antibodies having an activity against the Rhesus D antigen. Suitable expression systems for such antibodies are mouse myeloma cells or chinese hamster ovary cells.

The examples which follow explain the invention in detail, without any restriction of the scope of the invention.

Example 1 describes the construction of 2 combinatorial librairies; especially the aforementioned LD1 and LD2 libraries.

Example 2 describes a series of pannings using R1R1 rbc on the said LD1 and LD2 libraries in detail.

Example 3 describes a series of pannings using both bromelase and non bromelase treated rbc for absorption and bromelase treated DVI positive rbc using a pool of the said LD1 and LD2 librairies.

Example 4 describes an indirect haemagglutination assay using a rabbit anti-phage antibody, as an equivalent Coombs reagent, to monitor the enrichment and specificity of Rhesus D specific Phabs after panning.

Example 5 describes the preparation and purification of Fab antibody fragments for application as diagnostic reagents.

Example 6 describes the preparation of complete anti-Rhesus D immunoglobulins using the sequences of the present invention.

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Example 1

Construction of the recombinant LD1 and LD2 libraries

a) Source of the lymphocytes

A male adult who was a member of the volunteer pool of
hyperimmune Rhesus D donors was given an i.v. boost of 2 ml of packed rbc
from a known male donor of blood group O RhD*. The PBL were harvested at
+5 and +18 days after the boost and the mononuclear cells (MNC) isolated by
Ficoll gradient centrifugation (Lymphoprep, Pharmacia, Milwaukee, WI). The
results of donor lymphocyte analysis of day +5 are given in table 4. The +5
day MNC were used directly for RNA preparation using a phenol-chloroform
guanidinium isothiocyanate procedure (Chomczynski, P. and Sacchi, N.,
Anal. Biochem. 162:156, 1987). The +18 day MNC were first cultured for 3
days in RPMI-1640 medium (Seromed, Basel) containing 10³ U/ml of IL-2
(Sandoz Research Center, Vienna, Austria) and 10 μg/ml of pokeweed
mitogen (PWM; Sigma L9379, Buchs, Switzerland) before extracting RNA.

Immunofluorescence analysis of donor lymphocytes +5 days after rbc i.v. boost

Cell surface antigen	% Positive cells	Cell surface antigen	% Positive cells	
CD20	15	CD8	12	
CD38	20	CD25	7.6	
CD20/38	15	CD57	12.5	
CD20/30	47	CD14	6	
	34	HLA-DR	18	
CD4		1,2,7,0,1		

b) Construction of Library

Two separate libraries were constructed called LD1 and LD2 (as detailed above) corresponding to the cells harvested at +5 days and +18 days (finally +21 days including the +3 days PWM stimulation) after the i.v. boost respectively. Total RNA was then prepared from these cells using a phenol-chloroform guanidinium isothiocyanate method. From this RNA, 10 µg were

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used to make cDNA using an oligo(dT) primer (400 ng) and reverse transcribed with M-MuLV reverse transcriptase according to the conditions specified by the supplier (Boehringer Mannheim Germany). PCR amplification was performed as described in Vogel, M. et al., E.J. of Immunol. 24:1200, 1994. Briefly, 100 μl PCR reaction contained Perkin-Elmer buffer with 10 mM MgCl₂, 5 μl cDNA, 150 ng of each appropriate 5' and 3' primer, all four dNTP at 200 μM each and 2 U/ml Taq Polymerase (Perkin Elmer, NJ). The PCR amplification of the heavy and light chains of the Fab molecule was performed separately with a set of primers from Stratacyte (details given below). For the heavy chain six upstream primers were used that hybridize to each of the six families of the V_{H} genes whereas one kappa and one lambda chain primer were used for the light chain. The downstream primers were designed to match the hinge region of the constant domains $\gamma 1$ and $\gamma 3$ for the heavy chain. For the light chain the downstream primers were matched to the 3^{\prime} end of kappa and lambda constant domains. The heavy and light chain PCR products were pooled separately, gel purified and cut with Xho1/Spe1 and Sac1/ Xba1 restriction enzymes (Boehringer Mannheim), respectively. After digestion the PCR products were extracted once with phenol: chloroform: isoamylalcohol and purified by gel excision. The insertion of the Xho1/Spe1 digested Fd fragment and subsequent ligation of the Sac1/Xba1 digested light chain into the pComb3 vector, the transformation into XL1-Blue cells, and the production of phages were performed as described by (Barbas III, C.F. and Lerner, R.A., Companion Methods Enzymol. 2:119, 1991).

After transformation of the XL1-Blue E.coli cells samples were withdrawn and titrated on plates to determine the library size. These results indicated expression libraries of 7.5×10^6 and 7.7×10^6 cfu (colony forming units) for LD1 and LD2 respectively.

c) PCR Primers

VHI 5'-CAC TCC CAG GTG CAG CTG CTC GAG TCT GG-3'

VHII 5'-GTG CTG TCC CAG GTC AAC TTA CTC GAG TCT GG-3'

VHIII 5'-GTC CAG GTG GAG GTG CAG CTG CTC GAG TCT GG-3'

VHIV 5'-GTC CTG TCC CAG GTG CAG CTG CTC GAG TCG GG-3'

VHV 5'-GTC TGT GCC GAG GTG CAG CTG CTC GAG TCT GG-3'

VHVI 5'-GTC CTG TCA CAG GTA CAG CTG CTC GAG TCA GG-3'

CHI(gI) 5'-AGC ATC ACT AGT ACA AGA TTT GGG CTC-3'

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- VL(k) 5'-GT GCG AGA TGT GAG CTC GTG ATG ACC CAG TCT GAA GCT
- CL(k) 5'-T CCT TCT AGA TTA CTA ACA CTC TCC CCT GTT GAA GCT CTT TGT GAC GGG CGA ACT C-3'
- VL(I) 5'C TGC ACA GGG TCC TGG GCC GAG CTC GTG GTG ACT CA-3'
- 5 CL(I) 5'G CAT TCT AGA CTA TTA TGA ACA TTC TGT AGG GGC-3'

d) Vectors and bacterial strains

The pComb3 vector used for cloning of the Fd and the light chain was obtained from the Scripps Research Institute La Jolla, CA; (Barbas III, C.F. and Lerner, R.A., Companion Methods Enzymol. 2:119, 1991). The *Escherichia coli* strain XL1-Blue used for transformation of the pComb3 vector and the VCSM13 helper phage were purchased from Stratacyte (La Jolla, CA).

Example 2

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Selection of Rhesus D Phabs from LD1 and LD2 libraries on R1R1 rbc

a) Absorption and Bio-Panning

A series of three negative absorptions on rbc group O Rh negative were performed for each panning round before positive selection on rbc group O Rh positive (R1R1). Fresh rbc were collected in ACD (acid citrate dextrose) anticoagulant and washed 3 times in 0.9% NaCl. The rbc were counted in Hayems solution and adjusted to 40x10⁶/ml. Absorption: 1 ml of phage preparation in PBS/3%BSA was added to rbc group O Rh negative pellet (16x10⁶ rbc) in 12 ml tubes (Greiner 187261, Reinach, Switzerland) and incubated at RT for 30 min. with careful shaking. All tubes were pre-blocked in PBS/3% BSA for a minimum of 1hr at RT. The rbc were pelleted by centrifuging for 5 min. 300 x g at 4°C. The resulting phage supernatant was carefully harvested and the process repeated twice more. After the final absorption the phage supernatant was added to the rbc group O Rh positive pellet (16x10 rbc) and again incubated at RT for 30 min. with gentle shaking. Then the rbc were washed at least 5 times in 10 ml ice cold PBS, centrifuged 5 min. 300 x g at 4°C, followed by elution with 200 μ l of 76 mM citric acid pH 2.8 for 6 min. at R.T. and neutralisation with 200 μ l 1M Tris. The rbc were centrifuged 300 x g, 5 min. at 4°C and the resulting supernatant containing the eluted phages was carefully removed and stored with carrier protein

(0.3% BSA) at 4°C ready for re-amplification. The numbers of Rhesus D specific Phabs of each panning round are given in table 5.

Table 5

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Selection of Rhesus D+ Phabs from the LD1 and LD2 libraries on R1R1 rbc

	No. of eluted Rhesus D specific phages				
Panning Round No. ^{a)}	Library D1 cfu	Library D2 cfu			
1	8x10 ⁶	4.6x10 ⁷			
2	6x10 ⁷	1.4x10 ⁷			
3	1x10 ⁸	7.9x10 ⁷			
4	3x10 ⁸	1.3x10 ⁸			
5	3x10 ⁸	1x10 ⁸			
6	nd	2.8x10 ⁸			

a) For each round 10¹² Phabs were incubated in tubes with rbc Group O Rhesus negative (absorption phase) followed by elution from rbc Group O Rhesus positive (R1R1)

nd = not done

cfu = colony forming units

Example 3

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Selection of Rhesus D Phabs from the pooled LD1 and LD2 libraries on DVI+ rbc

a) Absorption on rbc group O Rh negative, phenotypes

1 (r'r, Ccddee) and 2 (ryry, CCddEE)

A series of four negative absorptions on rbc group O Rh negative was performed for each panning round before positive selection on rbc group O Rh DVI positive. The negative absorptions were performed in the following order: Step 1) phenotype 1 treated with bromelase; step 2) phenotype 1 no bromelase; step 3) phenotype 2 treated with bromelase; step 4) phenotype 2

no bromelase. Frozen rbc were thawed into a mixture of sorbit and phosphate buffered saline, left standing in this solution for a minimum of 10 min. and then washed 5 to 6 times in phosphate buffered saline and finally stored in stabilising solution (DiaMed EC-Solution) ready for use. Before panning the rbc were washed 3 times in 0.9% NaCl. followed by counting in Hayems solution. Absorption: 1 ml of phage preparation in PBS/3%BSA was added to an rbc pellet (2x10⁸) as in step 1 in 12 ml tubes (Greiner 187261, Reinach, Switzerland) and incubated at RT for 30 min. with careful shaking. All tubes were pre-blocked in PBS/3% BSA for a minimum of 1hr at RT. The rbc were pelleted by centrifuging for 5 min. 300 x g at 4°C. The resulting phage supernatant was carefully harvested and the process repeated using rbc as detailed above in steps 2, 3, and 4.

b) Treatment of rbc Rhesus D negative r'r and ryry and Rhesus DVI+ with bromelase

Bromelase 30 (Baxter, Düdingen, Switzerland) was used to treat rbc Rhesus DVI+ in the same proportions as used in a routine haemagglutination assay, i.e. 10 µl bromelase per 2x10⁶ rbc. Thus bromelase was added to the required amount of rbc and incubated at 37°C for 30 min. followed by washing 3 times in 0.9% NaCl, re-counting in Hayems solution and adjusting to the required concentration in PBS/3% BSA ready for Phab panning.

c) Bio-Panning on bromelase treated Rhesus DVI+ rbc

After the final absorption on rbc ryry non bromelase treated the phage supernatant was divided into 2 equal parts and added either to the enzyme or non enzyme treated rbc group O Rh DVI+ pellet (40×10^6) respectively and again incubated at RT for 30 min. with gentle shaking. Then the 2 populations of rbc were washed at least 5 times in 10 ml ice cold PBS, centrifuged 5 min. $300 \times g$ at $4^{\circ}C$, followed by elution with $200 \ \mu l$ of 76 mM citric acid pH 2.8 for 6 min. at R.T. and neutralisation with $200 \ \mu l$ 1M Tris. The rbc were centrifuged $300 \times g$, 5 min. at $4^{\circ}C$ and the resulting supernatants containing the eluted phages from either the bromelase or non bromelase treated DVI+rbc were carefully removed and stored with carrier protein (0.3% BSA) at $4^{\circ}C$ ready for re-amplification. In further rounds of panning the eluted phage from either the bromelase treated DVI+ rbc were

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kept separate and each followed the absorption protocol steps 1 to 4. The elution step was slightly different compared to panning round 1 as the phage populations were not again divided into 2 parts. Only those phage eluted from bromelase treated DVI+ rbc were also eluted again from bromelase treated DVI+ rbc and only those phage eluted from the non bromelase treated DVI+ rbc were also again eluted from non bromelase treated DVI+ rbc. The numbers of specific Phabs after each panning round are given in table 6.

Table 6 Selection of Rhesus D Phabs from pooled LD1 and LD2 libraries on Rhesus DVI+ red blood cells

	No. of eluted Rhesus DVI+ specific phages				
Panning Round No. ^a)	- Bromelase cfu	+ Bromelase cfu			
1	1.9x10 ⁶	4.4x10 ⁶			
2	1.6x10 ⁶	4x10 ⁵			
3	2.4x10 ⁷	4.1x10 ⁷			
4	3x10 ⁶	5x10 ⁷			
5	1x107 ⁸	1×10 ⁸			
6	nd	3x10 ⁸			

a) For each round 10¹² Phabs were incubated in tubes with 2 different phenotypes of rbc Group O Rhesus negative (absorption phase) followed by elution from rbc Group O Rhesus DVI+.

Example 4

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Monitoring of the panning rounds and determination of the specificity of the enriched Phabs using a rabbit anti-phage antibody

Indirect haemagglutination assay

Freshly collected rbc of different ABO and Rhesus blood groups were washed 3 times in 0.9% NaCl and adjusted to a 3-5% solution (45- $50x10^7$ /ml) in either 0.9% NaCl or PBS/3% BSA. For each test condition $50~\mu l$ rbc and 100 μl test (precipitated and amplified phage or control antibodies) were incubated together in glass blood grouping tubes (Baxter, Düdingen, Switzerland) for 30 min. at 37°C. The rbc were washed 3 times in 0.9% NaCl

and then incubated with 2 drops of Coombs reagent (Baxter, Düdingen, Switzerland) for positive controls or with 100 µl of 1/1000 diluted rabbit antiphage antibodies (made by immunising rabbits with phage VCSM13 preparation, followed by purification on an Affi-Gel Blue column and absorption on E. coli to remove E. coli-specific antibodies). The tubes were incubated for 20 min at 37°C, centrifuged 1 min at 125xg and rbc examined for agglutination by careful shaking and using a magnifier viewer.

When purified Fab were tested for agglutination, an affinity purified anti-Fab antibody (The Binding Site, Birmingham, U.K.) was used instead of the rabbit anti-phage antibody.

Table 7 shows the results of haemagglutination tests of Phab samples after different panning rounds on R1R1 rbc.

Table 8 shows the results of haemagglutination tests of Phab samples after different panning rounds on Rhesus DVI+ rbc.

Table 9 shows the reactivity pattern of individual Fab clones from libraries LD1 and LD2 with partial D variants.

Table 7 Monitoring of Phabs from LD1 and LD2 libraries by indirect haemagglutination after panning on R1R1 rbc

haemagglutination after pariting of the same and the same							
Phab sample	Library LD1	Library LD2					
Panning round	tested on rbo	O Rh D+ (a)					
No. 4							
undiluted	+	+/-					
1/4	+	7/-					
1/20	-	-					
No.5		_					
undiluted	++	T					
1/4	++	•					
1/20	-	-					
No. 6		+++					
undiluted	nd	++					
1/4	nd	nd					
1/20	nd	Tiu					
Helper phage (b)		_					
undiluted, 1/4, 1/20							

 $_{20}$ a) Indirect haemagglutination was performed in glass tubes using 50 μl rbc $(40x10^7/ml)$ and 100 μl Phabs starting at 4x10 $^{11}/ml$. After 30 min. at 37°C the

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rbc were washed 3 times and further incubated for 20 min. at 37°C with a 1/1000 dilution of rabbit anti-phage antibody.

- b) The M13 helper phage was used as a negative control and showed no non-specific agglutination due to the phage particle alone.
- Agglutination was scored by visual assessment from +++ (strong agglutination) descending to (no agglutination). nd = not done

Table 8 Monitoring of Phabs from pooled LD1 and LD2 libraries by indirect haemagglutination after panning on Rhesus DVI+ rbc

Phab sample			rbc ph	nenotypes		
Panning round						
	CCDDee	ccddee	Ccddee	CCddEE	DVI (E.J.)	DVI (K.S.)
Non Bromelase						
treated rbc DVI+						
Round No.3	a) +++	-	+/-	(+)	+/-	+/-
Round No. 5	++	_	-	•	•	• •
Bromelase						
treated rbc DVI+						
Round No.4	+++	-	+/-	_	(+)	+/-
Round No.5	+++	•	+/-	+/-	(+++)	++
Round No.6	++++	-	-	-	+++	+++
LD1 - 6 - 17			reactive v	with C and I		
LD1/2 - 6 - 3	++++	-	-	-	+/-	nd
LD1/2 - 6 - 33	++++	-	-	-	+	nd

a) Agglutination was scored by visual assessment from ++++ (strong agglutination) descending to - (no agglutination). nd = not done Note: Only those Phabs eluted from bromelase treated DVI+ rbc showed evidence of agglutination against 2 different DVI+ donors.

Table 9

Clonal Analysis of Reactivity of Fab anti-Rh sus D Clones from Libraries

D1 and LD2 against Partial D Variants

			Parti	al D Vari	iants		
^(a) Fab Clone No	Rh33	DIII	DIVa	DIVb	DVa	DVI	DVII
LD1 - 40		(b)+++	+	+	+/-	-	++
- 52	_	+++	-	-	+++	-	+++
- 84	_	++	-	-	-	-	+
- 110	(+)	+++	++	+	+	-	++
- 117	-	+++	-	-	-	-	++
LD2 - 1	+++	nd	+++	+++	+	-	+++
- 4	-	+++	-	+	-	-	+
- 5	-	nd	+++	+++	-	-	+++
- 10	(-)	+++	+++	+++	+	-	++
- 11	-	+++	-	-	-	-	++
- 14	+++	+++	+++	+++	+++	-	+++
- 17	-	+++	+++	+	+/-	-	+++
- 20	_	+++	+++	-	+/-	-	+++
LD1/2 - 6- 3	++	+++	+++	++	+++	+	++
LD1/2 - 6- 33	+/-	+++	+++	++	+++	+	++

a) soluble Fab preparations were made of each clone followed by indirect haemagglutination.

b) Agglutination was scored by visual assessment from +++ (all cells agglutinated in a clump) descending to - (no cells agglutinated).

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Example 5

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Preparation and purification of Fab antibody fragments for application as diagnostic reagents

After the bio-panning procedures detailed in Examples 2 and 3 a phage population which showed specific agglutination on Rhesus D+ rbc was selected and used to prepare phagemid DNA. More precisely the Phabs selected on R1R1 rbc were used after the 5th and 6th rounds of bio-panning for LD1 and LD2 libraries respectively and after the 5th bio-panning on DVI+ rbc for isolation of the LD1-6-17 clone. In order to produce soluble Fab, the sequence gIII coding for the pIII tail protein of the phage particle must be deleted.

Phagemid DNA was prepared using a Nucleotrap kit (Machery-Nagel) and the glll sequence was removed by digesting the so isolated phagemid DNA with Nhe1/Spe1 as described (Burton, D.R., et al., PNAS, 1989). After transformation into XL1-Blue individual clones were selected (nomenclature given in table 1) and grown in LB (Luria Broth) containing 50 μ g/ml carbenicillin at 37°C to an OD of 0.6 at 600 nm. Cultures were induced with 2 mM isopropyl β-D-thiogalactopyranoside (IPTG) (Biofinex, Praroman, Switzerland) and grown overnight at 37°C. The whole culture was spun at 10,000xg for 30 min. at 4°C to pellet the bacteria. The bacterial pellet was treated with a lysozyme/DNase solution to liberate the Fab fragments inside the cells. As some Fab were released into the culture supernatant this was also harvested separately. These Fab preparations were then pooled and precipitated with 60% ammonium sulphate (Merck, Darmstadt, Germany) to concentrate the Fab followed by extensive dialysis in phosphate buffered saline (PBS) and ultracentrifugation at 200,000xg to pellet any insoluble complexes. The Fab preparations were then purified on a ceramic hydroxyapatite column (HTP Econo cartridge, BioRad, Glattbrugg, Switzerland) using a gradient elution of PBS (Buffer A) and PBS + 0.5M NaCl (Buffer B). The linear gradient was programmed to increase from 0-100% Buffer B in 40 min. The Fab was eluted as a single peak between 40-60% Buffer B. The positive fractions as identified by immunodot assay using an anti-Fab peroxidase conjugate (The Binding Site, Birmingham, U.K.) were pooled, concentrated using polyethylene glycol and extensively dialysed

against PBS. The positive fractions from the hydroxyapatite column for each clone were used in a classical indirect haemagglutination assay in glass tubes using either the standard Coombs reagent (Baxter Diagnostics AG Dade, anti-human serum) or an anti-Fab (The Binding Site, Birmingham, U.K.) as the cross linking reagent. These Fab of defined specificity on the Partial D variants as shown on page 18 can be used to type rbc of unknown Partial D phenotype.

Example 6

Construction of complete immunoglobulin genes

The LD2-14 heavy chain V gene (V_H gene) was amplified from the anti-Rhesus D-Fab-encoding plasmid LD2-14 with the polymerase chain reaction (PCR) using specific primers. The 5'-primer had the sequence: 5'-GGGTCGACGCACAGGTGAAACTGCTCGAGTCTGG-3', whereas the 3'-primer was of the sequence:

15 5'-GCCGATGTGTAAGGTGACCGTGGTCCCCTTG-3'.

The PCR reaction was performed with Deep Vent DNA Polymerase and the buffer solution (2mM Mg**) from New England Biolabs at the conditions recommended by the manufacturer including 100 pmol of each primer and the four deoxynucleotides at a concentration of 250 μM each. The reaction was run for 30 cycles with the following temperature steps: 60 s at 94°C (extended by 2 min. during the first cycle), 60 s at 57°C and 60 s at 72°C (extended by 10 min. during the last cycle). Post-amplification addition of 3' A-overhangs was accomplished by a subsequent incubation for 10 min at 72°C in the presence of 1 unit Taq DNA Polymerase (Boehringer Mannheim, Germany). The PCR product was purified using the QIAquick PCR purification kit (Qiagen, Switzerland) and cloned into the vector pCRII using Invitrogen's TA cloning kit (San Diego, USA). Having digested the resulting plasmid TAVH14 with Sall and BstEll, the V_H gene was isolated by preparative agarose gel electrophoresis using Qiagen's QIAquick gel extraction kit. 30

Vector # 150 (Sandoz Pharma, Basel) which contained an irrelevant but intact human genomic immunoglobulin $V_{\rm H}$ gene was cut with

Sall and BstEll, and the vector fragment was isolated by preparative agarose gel electrophoresis using Qiagen's QIAquick gel extraction kit. Ligation of vector and PCR product was performed at 25°C for 2 hours in a total volume of 20 μl using the rapid DNA Ligation kit (Boehringer Mannheim, Germany).

Following ligation, the reaction mix was diluted with 20 μl H₂0 and extracted with 10 volumes of n-butanol to remove salts. The DNA was then pelleted by centrifugation, vacuum dried and resuspended in 10 μl H₂0. 5 μl of this DNA solution were electroporated (0.1 cm cuvettes, 1.9 kV, 200 Ω, 25 μFD) with a GenePulser (BioRad, Gaithersburg) into 40 μl of electroporation competent E. coli XL1-blue MRF' (Stratagene, La Jolla), diluted with SOC medium, incubated at 37°C for 1 hour and plated on LB plates containing ampicillin (50 μg/ml). Plasmid-minipreps (Qiagen, Basel) of the resulting colonies were checked with restriction digests for the presence of the appropriate insert.

With this procedure, the irrelevant resident V_H gene in vector # 150 was replaced by the amplified anti-Rhesus D V_H sequence of LD2-14 and yielded plasmid cassVH14. The structure of the resulting immunoglobulin V_H gene construct was confirmed by sequencing, cut out by digestion with *EcoRI* and *Bam*HI and gel purified as described above. Expression vector # 10 (Sandoz Pharma, Basel) containing the human genomic immunoglobulin Cγ1 gene segment was also digested with *EcoRI* and *Bam*HI, isolated by preparative agarose gel electrophoresis, ligated with the *EcoRI I Bam*HI-V_H gene segment previously obtained from plasmid cassVH14 and electroporated into E. coli XL1-blue MRF' as outlined above. This resulted in a complete anti-Rhesus D heavy chain immunoglobulin gene in the expression vector 14IgG1 (Figure and).

The LD2-14 light chain V gene (V_L gene) was amplified from the same anti-Rhesus D-Fab plasmid LD2-14 by PCR using specific primers. The 5'-primer had the sequence:

5'-TACGCGTTGTGACATCGTGATGACCCAGTCTCCAT-3', whereas the 3'-primer was of the sequence:

5'-AGTCGCTCAGTTCGTTTGATTTCAAGCTTGGTCC-3'.

PCR reaction, product purification and subsequent cloning steps were analogous to the steps described for the V_H gene, except that the appropriate light chain vectors were used. Briefly, the V_L PCR product was

cloned into pCRII vector yielding plasmid TAVL14, excised therefrom with Mlul and $\mathit{HindIII}$ and isolated by gel extraction. The V_L gene was subsequently cloned into the Mlul and $\mathit{HindIII}$ sites of vector # 151 (Sandoz Pharma, Basel) thus replacing the irrelevant resident V_L gene by the amplified anti-Rhesus D V_L sequence of LD2-14. Having confirmed the sequence of the resulting plasmid cassVL-14, the EcoRII Xbal fragment containing the V_L gene was then subcloned into the restriction sites EcoRI and Xbal of vector # 98 (Sandoz Pharma, Basel, Switzerland) which contains the human genomic immunoglobulin C_K gene segment. This procedure replaced the irrelevant resident V_L gene in plasmid # 98 and yielded the expression vector 14kappa which contains the complete anti-Rhesus D light chain immunoglobulin gene.

The mouse myeloma cell line SP2/0-Ag 14 (ATCC CRL 1581) was cotransfected by electroporation with the expression vectors 14lgG1 and 14kappa previously linearized at the unique EcoRI and NotI cleavage site, respectively. The electroporation was performed as follows: exponentially growing cells were washed twice and suspended in phosphate buffered sucrose (272 mM sucrose, 1 mM MgCl₂, 7 mM NaH₂PO₄, pH 7.4) at a density of 2 x 10⁷ cells/ml. 0.8 ml of cells were added to a 0.4 cm cuvette, mixed with 15 μg of linearized plasmids 14lgG1 and 14kappa, held on ice for 15 min., electroporated with 290 Volts, 200 Ω , 25 μ FD, put back on ice for 15 min., transferred to a T75 cell culture flask with 20 ml of cold RPMI 1640 medium (10% heat inactivated fetal bovine serum, 50 μM beta-mercaptoethanol), left for 2 h at room temperature and then incubated for 60 h at 37°C. After this period, the cells were transferred to 50 ml of medium containing 1 mg/ml G418 for selection. Stable transfectants were then selected in the presence of increasing concentrations of methotrexate to amplify the integrated DNA and thus increasing the expression of the corresponding antibody rD2-14.

Expression of rD2-14 in the culture's supernatant (SrD2-14) was monitored by an enzyme linked immuno-sorbent assay (ELISA) specific for human $\gamma 1$ and kappa chains. Quantification of the Rhesus D specific immunoglobulins in the anti-D assay according to Ph. Eur. revealed between 1.1 and 11.4 μ g/ml of agglutinating antibody in such supernatants. They tested agglutination negative for Rhesus negative rbc and revealed the same agglutination potential against partial D variants as the Fab LD2-14 expressed in E. coli. The data are shown in table 10.

Table 10

Comparativ analysis of reactivity of Fab anti-Rhesus D clone LD2-14

and antibody rD2-14 against partial D variants

				Partial D Variants					
	R1R1	rr	Rh33	DIII	DIVa	DIVb	DVa	DVI	DVII
LD2-14	+++	_	+++	+++	+++	+++	+++	-	+++
SrD2-14	+++	-	+++	+++	+++	+++	+++	-	+++
ТСВ	-	-							

Agglutination was scored by visual assessement from +++ (all cells agglutinated in a clump) descending to - (no cells agglutinated).

LD2-14: Fab fragment prepared as described in Example 5;

SrD2-14: cell culture supernatant containing antibody rD2-14;

TCB: cell culture supernatant of untransfected cells.

Claims

1. Polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens which include Rhesus D-specific CDR 1, CDR 2 and CDR 3 regions of pairs of amino acid sequences V_H and V_L with the same or different identification numbers according to the figures given in the table below:

			'н		VL				
Identi- fication	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.	
No. LD1-40	Fig. la	91-105	148-198	295-342	Fig. 1b	64-96	142-162	259-288	
LD1-52	Fig. 2a	91-105	148-198	295-342	Fig. 2b	64-96	142-162	259-288	
	Fig. 3a	91-105	148-198	295-342	Fig. 3b	64-96	142-162	259-285	
LD1-84		91-105	148-198	295-342	Fig. 4b	64-96	142-162	259-285	
LD1-110	Fig. 4a	91-105	148-198	295-345	Fig. 5b	64-96	142-162	259-288	
LD1-117	Fig. 5a	91-105	148-198	295-342	Fig. 6b	61-99	145-165	262-294	
LD2-I	Fig. 6a		148-198	295-342	Fig. 7b	64-96	142-162	259-282	
LD2-4	Fig. 7a	91-105	148-198	295-342	Fig. 8b	64-96	142-162	259-288	
LD2-5	Fig. 8a	91-105	1	298-345	Fig. 9b	61-102	148-168	265-294	
LD2-10	Fig. 9a	91-105	148-198		Fig. 10b	 	142-162	259-285	
LD2-11	Fig. 10a	91-105	148-198	295-342		+	142-162	259-285	
LD2-14	Fig. 11a	91-105	148-198	295-342	Fig. 11b	 	142-162	259-285	
LD2-17	Fig. 12a	91-105	148-198	295-342	Fig. 12b		142-162	259-285	
LD2-20	Fig. 13a	91-105	148-198	295-342	Fig. 13b			259-285	
LD1-6-17	Fig. 14a	91-105	148-198	295-351	Fig. 14b		142-162	+	
LD1/2-6-3	Fig. 15a	91-105	148-198	295-342	Fig. 15b		142-162	259-285	
LD1/2-6-33	Fig. 16a	91-105	148-198	295-342	Fig. 16b	64-96	142-162	259-285	

- 2. Polypeptides according to claim 1 which include Rhesus D-specific CDR 1, CDR 2 and CDR 3 regions of pairs of amino acid sequences $V_{\rm H}$ and $V_{\rm L}$ with the same identification numbers according to the figures given in the table of claim 1.
- 3. Polypeptides according to claim 1 which include regions with the amino acid sequences $V_{\rm H}$ and $V_{\rm L}$ and have identification numbers according to the figures given in the table of claim 1.

- 4. Polypeptides according to claim 1, 2 or 3 characterised as antigen binding Fab fragments.
- 5. Polypeptides according to claim 1, 2 or 3 comprising immunoglobulin heavy and light chains capable of forming complete anti-Rhesus D antibodies.
- 6. DNA sequences coding for polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens which include regions with the Rhesus D-specific CDR 1, CDR 2 and CDR 3 segments of pairs of DNA sequences V_H and V_L with the same or different identification numbers according to the figures given in the table below and functional equivalents thereof:

			/н				V _L	
Identi- fication No.	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.
LD1-40	Fig. la	91-105	148-198	295-342	Fig. 1b	64-96	142-162	259-288
LD1-52	Fig. 2a	91-105	148-198	295-342	Fig. 2b	64-96	142-162	259-288
LD1-84	Fig. 3a	91-105	148-198	295-342	Fig. 3b	64-96	142-162	259-285
LD1-110	Fig. 4a	91-105	148-198	295-342	Fig. 4b	64-96	142-162	259-285
LD1-117	Fig. 5a	91-105	148-198	295-345	Fig. 5b	64-96	142-162	259-288
LD2-1	Fig. 6a	91-105	148-198	295-342	Fig. 6b	61-99	145-165	262-294
LD2-4	Fig. 7a	91-105	148-198	295-342	Fig. 7b	64-96	142-162	259-282
LD2-5	Fig. 8a	91-105	148-198	295-342	Fig. 8b	64-96	142-162	259-288
LD2-10	Fig. 9a	91-105	148-198	298-345	Fig. 9b	61-102	148-168	265-294
LD2-11	Fig. 10a	91-105	148-198	295-342	Fig. 10b	64-96	142-162	259-285
LD2-14	Fig. 11a	91-105	148-198	295-342	Fig. 11b	64-96	142-162	259-285
LD2-17	Fig. 12a	91-105	148-198	295-342	Fig. 12b	64-96	142-162	259-285
LD2-20	Fig. 13a	91-105	148-198	295-342	Fig. 13b	64-96	142-162	259-285
LD1-6-17	Fig. 14a	91-105	148-198	295-351	Fig. 14b	64-96	142-162	259-285
LD1/2-6-3	Fig. 15a	91-105	148-198	295-342	Fig. 15b	64-96	142-162	259-285
LD1/2-6-33	Fig. 16a	91-105	148-198	295-342	Fig. 16b	64-96	142-162	259-285

7. DNA sequences according to claim 6 coding for polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens which include regions with the Rhesus D-specific CDR 1, CDR 2 and CDR 3 segments of pairs of DNA sequences V_H and V_L with the same

identification numbers according to the figures given in claim 6, and functional equivalents thereof.

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- 8. DNA sequences according to claim 6 or 7 which include regions with the DNA sequences $V_{\rm H}$ and $V_{\rm L}$ with the identification numbers according to the figures given in claim 6.
 - 9. DNA sequences according to claim 6, 7 or 8 coding for polypeptides capable of forming antigen binding Fab fragments.
 - 10. DNA sequences according to claim 6, 7 or 8 coding for polypeptides capable of forming complete anti-Rhesus D antibodies.
- 11. A process for preparing recombinant polypeptides capable of forming antigen binding structures, e.g. Fab fragments, with specificity for Rhesus D antigens which process comprises the following steps in sequential order:
 - a) boosting of an individual capable of forming anti-Rhesus D antibodies with Rhesus D positive red blood cells,
 - b) isolating mononuclear cells from the individual,
 - c) isolating total RNA from the mononuclear cells,
 - d) preparing a cDNA by using an oligo(dT)primer and reverse transcribing of the mRNA with M-MuLV reverse transcriptase and amplifying the cDNA repertoire by a polymerase chain reaction using immunoglobulin gene family specific primers,
 - e) creating a phage display library by inserting the DNA coding for the heavy and light chain of the Fab polypeptide into a phagemid vector; the DNA for the heavy chain is inserted in frame to the gene coding for the phage protein pIII which allows the expression of a Fab pIII fusion protein on the surface of the phage,
 - f) transforming bacterial cells with the obtained recombinant plasmids, cultivating of the transformed bacterial cells and co-expression of the heavy and the light chain of a Fab on filamentous phage particles,

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- g) amplifying the Fab-carrying phage in bacteria,
- h) selecting individual phage clones by several rounds of panning on Rhesus positive red blood cells.
- i) isolating the plasmid DNA from the selected clones and cutting out the gIII gene,
- transforming bacterial cells with the obtained plasmid, cultivating of the transformed bacterial cells expressing the Fab, and isolating the Fab fragments.
- 12. A process for selecting recombinant polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens and in particular showing reactivity with the Partial Rhesus DVI Variant and without any evidence of reactivity with red blood cells of Rhesus negative phenotypes in particular without reactivity against the Rhesus alleles C, c, E, and e which process comprises the following steps in sequential order:
- a) performing several negative absorptions on the following red blood cells: phenotype 1 (r'r, Ccddee) treated with bromelase, phenotype 1 not treated with bromelase, phenotype 2 (ryry, CCddEE) treated with bromelase and phenotype 2 not treated with bromelase,
 - b) performing a positive absorption on DVI+ red blood cells with or without bromelase treatment,
 - c) determining the titer of phage binding to DVI+ red blood cells
 - d) repeating steps a), b) and c) until the titer of phage binding to DVI+ red blood cells has reached a satisfactory level.
 - 13. A process according to claim 12, wherein the recombinant polypeptides capable of forming antigen binding structures are Fab fragments.
- 14. Anti-Rhesus D antibodies having heavy and light chain variable
 regions comprising the Rhesus D-specific CDR 1, CDR 2 and CDR 3

sequences of pairs of amino acid sequences V_{H} and V_{L} having the same or different identification numbers according to the table below:

			/н				V _L	
Identi- fication No.	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.
LD1-40	Fig. la	91-105	148-198	295-342	Fig. 1b	64-96	142-162	259-288
LD1-52	Fig. 2a	91-105	148-198	295-342	Fig. 2b	64-96	142-162	259-288
LD1-84	Fig. 3a	91-105	148-198	295-342	Fig. 3b	64-96	142-162	259-285
LD1-110	Fig. 4a	91-105	148-198	295-342	Fig. 4b	64-96	142-162	259-285
LD1-117	Fig. 5a	91-105	148-198	295-345	Fig. 5b	64-96	142-162	259-288
LD2-1	Fig. 6a	91-105	148-198	295-342	Fig. 6b	61-99	145-165	262-294
LD2-4	Fig. 7a	91-105	148-198	295-342	Fig. 7b	64-96	142-162	259-282
LD2-5	Fig. 8a	91-105	148-198	295-342	Fig. 8b	64-96	142-162	259-288
LD2-10	Fig. 9a	91-105	148-198	298-345	Fig. 9b	61-102	148-168	265-294
LD2-11	Fig. 10a	91-105	148-198	295-342	Fig. 10b	64-96	142-162	259-285
LD2-14	Fig. 11a	91-105	148-198	295-342	Fig. 11b	64-96	142-162	259-285
LD2-17	Fig. 12a	91-105	148-198	295-342	Fig. 12b	64-96	142-162	259-285
LD2-20	Fig. 13a	91-105	148-198	295-342	Fig. 13b	64-96	142-162	259-285
LD1-6-17	Fig. 14a	91-105	148-198	295-351	Fig. 14b	64-96	142-162	259-285
LD1/2-6-3	Fig. 15a	91-105	148-198	295-342	Fig. 15b	64-96	142-162	259-285
LD1/2-6-33	Fig. 16a	91-105	148-198	295-342	Fig. 16b	64-96	142-162	259-285

- 15. Anti-Rhesus D antibodies having heavy and light chain variable regions comprising the Rhesus D-specific CDR 1, CDR 2 and CDR 3 sequences of pairs of amino acid sequences V_H and V_L having the same identification numbers as indicated in the table of claim 14.
 - 16. Anti-Rhesus D antibodies according to claim 14 or 15 which include pairs of amino acid sequences V_{H} and V_{L} having the identification numbers according to the figures, as indicated in the table of claim 14.
- 17. Anti-Rhesus D antibodies according to claims 14, 15, or 16 wherein the immunoglobulin constant regions are of at least one of the defined isotypes IgG1, IgG2, IgG3 or IgG4.

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- 18. A process for preparing complete anti-Rhesus D antibodies according to one of the claims 14 to 17, comprising in sequential order the steps of
 - a) amplifying separately the members of a pair of a heavy chain V gene segment and a light chain V gene segment containing Rhesus D-specific CDR 1, CDR 2 and CDR 3 regions as depicted in Figs. 1a 16a and 1b 16b, respectively, from an anti-Rhesus D-Fab-encoding plasmid by carrying out a polymerase chain reaction with specific primers,
- b) preparing separately the genes of a complete anti-Rhesus D immunoglobulin heavy chain and a complete anti-Rhesus D immunoglobulin light chain in suitable plasmids containing the immunoglobulin constant region gene segments coding for either one of the human γ1, γ2, γ3 and γ4 heavy chains and for the human κ or λ light chain and transforming the obtained plasmids separately in suitable E. coli bacteria, and
 - c) cotransfecting the obtained plasmids into suitable eukaryotic host cells, cultivating of the cells, separating the non-transformed cells, cloning of the cultures, selecting the best producing clone, using it as a production culture and isolating the complete antibodies from the supernatant of the cell culture.
 - 19. A pharmaceutical composition comprising at least one polypeptide according to the definition of claim 1, 2 or 3 or at least one anti-Rhesus D antibody according to one of the claims 14 to 17 for the prophylaxis of haemolytic disease of the newborn, for the treatment of idiopathic thrombocytopenic purpura and mistransfusions of Rhesus incompatible blood.
 - 20. A diagnostic composition for Rhesus D typing comprising Fab fragments according to claim 4 or anti-Rhesus D antibodies according to one of the claims 14 to 17.

Fig. 1a

LD1-40-VH sequence

CAG	стс	9 444	стс	CTC	18 GAG	TCT	GGG	27 GGA	GGC	GTG	36 GTC	ÇAG	CCT	45 GGG	AGG	TCC	С
																	_
Q	V	K	L	L	Е	S	G	G	G	V	V	Q	P	G	R	S	
		63			72			81			90			99			1
AGA	CTC	TCC	TGT	ATA	GCG	TCT	GGA	TTC	ACC	CTC	AGG	AAT	TAT	GCC	ATG	CAC	7
R	Ľ,	s	C	I	A	S	G	F	T	L	R	N	Y	А			
								200			144	←		- CDF 153	R1 —	>]
GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGG	L35 CTG	GAG	TGG	GTG	GCA	GGT	ATA	TGG	TTT	
			-														-
V	R	Q	A	Þ	G	K	G	L	E	W			G	I —— :			
		171			180			189			198			207			4
GGA	AGT	AAC	AAA	AAC	TAT	GCA	GAC	TCC	GTG	AAG	GGC	CGA	TTC	ACC	ATC	TCC	Ž
G	s	N	K	N		Α	D	S	V	K			F	T	I	S	
		225			- CDF 234	₹2 —		243			252	•		261			:
GAC	AAT	TCC	AAG	AAC	ACG	CTG	TAT	CTG	CAA	CTG	AAC	AGC	CTG	AGA			
 D		- 	 К	N	T	 L	Y	 L	Q	L L	N	s	L	R	D	Ε	
		279			288			297									
ACG	GCT	GTG	TAT	TAT	TGT	GCG	AGA	GAG	CGA	GCA	GCA	CGT	GGT	ATT	TCT	AGG	
 T	 А		Y	 Y	<u></u>	Α	 R	E	R	Α	A	R	G	I	s	R	
		222			3 4 0			251			360	— CD	R3 —	369 GTC			
TAT	TAC	333 TAC	ATG	GAC	GTC	TGG	GGC	AAA	GGG	ACC	ACG	GTC	ACC	GTC	TCC	CCA	
										 T			T			P	
•	•	-		_													

2/34

Fig. 1b

LD1-40-VL sequence

		9	•		18			27			36			45			5
GTG	ATG														AGA	GTC	AC
v	M	T	Q	S	P	s	s	L	s	Α	s	V	G	D	R	v	T
ATC	ACT						AGC									CAG Q CAA Q CTC L AGT	
	 T	 C	 R	 А	5 S	 Q	 S	 I	 R	- 	н	 L		 W	 Y	Q	Q
		117	←		126			CDR.	1 —		144			153			16
AAA	CCA						TTG										
K	P	G	 K	Α	P	K	L	L	I	Υ							
		171			180			189			•						
GGC	GTC						GGC										
G	v	P	s	R	 F	s	G	-	G	S	G	A		F	т	L	T
ATC	GCC						GAT										
-	Α	s	L	Q	P	<u></u> Е	D	 F	A	T	Y	Y	C	Q	E	s	Y
AGT	AAT						GGC							315 ACT	AAA	3'	
s —			L				G	Q	G	т	R	L	E	T	ĸ		

Fig. 2a

LD1-52-VH sequence

CNC	CTG	9 ممم	ርፕር	ርፕር	18 GAG	тст	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCG	45 GGG	GGG	TCC	(
												 Q					
Q	V	K	L	L	Ł												
		63			72			81			90		m c m	99	N TO C	CNC	
AGA	CTC	TCC	TGT	GAA	GCG	TCT	GGA	TTC	GCC	CTC	AGA	AGT	TCT				
R	L	S	С	Ξ	A	s	G	F	Α	L	R	S	S	G	M	Н	
											3.4.4			CDR1			٠
CTC	CC-	117	ССТ	CCT	126 660	AAG	GGG	135 CTG	GAG	TGG	GTG	GCA	CTT	ATA	TGG	TTT	•
V	R	Q	А	P								A	4	(ייאחר		_
		171			180			189			198			207			
GGA	AGT	ATC	AGA	TCG	TAT	GCA	GAA	TCC	GTG	AAG	GGC	CGA	TTC	ACC	ATC	TCC	•
 G	 S		 R	 S	 Y	 A	E	S	V	К	G	R	F	Т	I	s	
					- CDF	32						•					
GAC	ACT	225 TCC	AAG	AAC	234 ACC	CTA	TAT	CTC	CAA	ATG	CGC	AGT	CTG	AGT	GCC	GAC	
												s					
D	T	S	r	N													
		279	_		288			297	220	ccc	306	cee	GGA	315 ATT	AGC		
												CGG					
T	А	V	Y	Y	С	A	R	D	K	Α	V	R	G	I	S	R	
					242			351			360	(SDR3	369			
AAC	TAT	333 TAC	ATG	GAC	GTC	TGG	GGC	AAA	GGG	ACC	ACG	GTC	ACC	GTC			
							G G								-		
IN	Ţ	1	1.1	L	•	••	-										

Fig. 2b

LD1-52-VL sequence

			9			18			27			36			45			54
5 '	GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC
	v	M	T	Q	s	P	S	s	L	s	A	S	V	G	D	R	v	Т
			63			72			81		~~~	90	mm n		99	m » m	C D C	108
	ATC	ACT	TGC	CGG	GC.A	AGT	CAG	AAC	ATT	ATC		TAT	TTA	AAT	166	1A1	V CAG	
	I	T	С	R	A						R			N	W	Y	Q	Q
									CDR	l —							V CAG Q CAA Q CTC L AGT	1.62
	AAG	CCA	117 GGG	AAA	GCC	126 CCT	AGG	CTC	135 CTG	ATC	TAT	GGT	GCG	TCC	ACT	TTG	CAA	162 AGT
		 p	- G		 A	P	 R	L	 L	I	Y	- - -	A	s	 T	L	z .	-
																	V CAG Q CAA Q CTC L AGT	
	GGG	GTC	171 CCA	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GGA	TCT	GGG 198	ACA	GAT				216 ACC
	 G		P	s	P.	F	s	G	s	- - -	s	G	T	D	Ē	T	L	т
			225			234		_	243			252	m) 0	m cm	261	G1.6) cm	270
	ATC	AGT	AGT	CTG	C'y'Y	CCT	GAA	GAT	TTT	GCA	ACT	TAC	TAC	TGT	CAA	CAG	AGT	TAC
	I	S	s	L	Q	P	E	D	F	A	T	Y	Y	С	Q	Q	S	Y
			270			200			207			306			315			
	CGT	ACC	CCT	CCA	TTC	ACT	TTC	GGC	CCT	GGG	ACC	AAA	GTG	GAG	_	AAA	3'	
	 R	 T		 P	 F	 T	F	 G	P	G		 К	v	E	I	K		
			CDE	3														

Fig. 3a

LD1-84-VH sequence

		9			18			27			36			45			54
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCG	GGG	GGG	TCC	CTC
Q		к	 L	 L	E	s	-	- 	G	V	v	Q	P	G	G	s	L
		63			72			81			90						
AGA	стс	TCC	TGT	GAA	GCG	TCT	GGA	TTC	ACC	CTC	AGA	AGT	TCT	GGC	ATG	CAC	TGG
	 L	 S	- 	 Е	 А	- S	G	F	T	L	R						
		117			126			135			144			153			162
GTC	CGC	CAG	GCT	CCT	GGC	AAG	GGG	CTG	GAG	TGG	GTG	GCA	CTT	ATA	TGG	TTT	GAT
v	 R	Q	Α	P	 G	-	G	L	E		V	A	L	I	W	F	D
CCA	እ <i>ር</i> ጥ	171	מכמ	ጥርር	180	GCA	GAA	189 TCC	GTG	AAG	198			207		TCC	216
	AG1	A1C	AGA														
G	s	I	R			Α					G	R	F	Т	I	S	н
GAC	ACT	225 TCC			234	CTA		243			252 CGC	AGT	CTG			GAC	
 D	 T	s	 К	N	- <u>-</u> -		Y	L	Q	М	R	S	L	S	Α	D	D
ACG	GCT	279 GTG	TAT	TAC	288 TGT	GCG	AGA	GAC	AAG	GCG	GTT	CGG	GGA	315 ATT	AGC		324 TAC
 T	 A	v	 Y	 Y	c	70.	 R	D	ĸ	Α	v	R	G	I	S	R	Y
AAC	TAT	333 TAC	ATG	GAC	342 GTC	TGG	GGC	351 AAA	GGG	ACC	360 ACG	GTC	ACC	369 GTC	TCC	TCA	3 '
			 М								T						
			R3 —														

Fig. 3b

LD1-84-VL sequence

	9			18			27			36			45			54
ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	ATA	GGA	GAC	AGA	GTC	ACC
 М	T	Q	S	P	S	\$	L	s	A	S	I	G	D	R	V	Т
	63			72			81			90			99			108
ACC	TGC	CGG	GCA	AGT	CAG	AGT	ATC	ATC	AGG	TAT	TTG	TAA	TGG	TAT	CAG	CAC
T	C	R	Α	s	Q	s	I	I	R	Y	L	N	W	Y	Q	ī
							— CD	R1 -								
	117			126			135			144			153			163
CCA	GGA	AAA	GCC	CCT	AAA	CTC	CTC	ATC	TTT	GCT	GCA	TCG	AAT	TTG	CAA	AC'
 p				 Р					 F	 А	 А	s	- 	L	Q	Т
-	0		••	-	• •	_	_	_	_				CDR2			
	171			100			180									21
CTC	7/1	TCC	NGG	ተጥር	АСТ	GGC	AGT	GGA	тст	GGG	ACA	GAT	TTC			-
GIC	CCA		700		701											
V	P	S	R	F	S	G	s	G	s	G	T	D	F	T	L	T
	225			234			243			252			261			27
AGT	GAC	CTG	CAG	CCT	GAG	GAT	TTC	GCA	ACT	TAC	TAC	TGT	CAA	CAG	AGT	TA
S	D	L	Q	P	Ε	D	F	A	Т	Y	Y	С	Q	Q	5	Y
	270			200			297			306			315			
7.00		TTTC	$T \cap A$	200 7777	GGC	cee	GGG	ACC	AGC	CTG	GAC	ATC	AAA	3 '		
				1	-	-00	555									
AGG													- - -			
	ACC T CCA P GTC V AGT	M T 63 ACC TGC T C 117 CCA GGA P G 171 GTC CCA V P 225 AGT GAC S D	M T Q 63 ACC TGC CGG T C R 117 CCA GGA AAA P G K 171 GTC CCA TCC V P S 225 AGT GAC CTG S D L	M T Q S ACC TGC CGG GCA T C R A 117 CCA GGA AAA GCC P G K A 171 GTC CCA TCC AGG V P S R AGT GAC CTG CAG S D L Q	ATG ACC CAG TCT CCA M T Q S P ACC TGC CGG GCA AGT T C R A S 117 126 CCA GGA AAA GCC CCT P G K A P 171 180 GTC CCA TCC AGG TTC V P S R F AGT GAC CTG CAG CCT S D L Q P 279 288	ATG ACC CAG TCT CCA TCC M T Q S P S ACC TGC CGG GCA AGT CAG T C R A S Q 117 CCA GGA AAA GCC CCT AAA P G K A P K 171 CCA TCC AGG TTC AGT V P S R F S AGT GAC CTG CAG CCT GAG S D L Q P E	ATG ACC CAG TCT CCA TCC TCC M T Q S P S S ACC TGC CGG GCA AGT CAG AGT T C R A S Q S 117 CCA GGA AAA GCC CCT AAA CTC P G K A P K L 171 GTC CCA TCC AGG TTC AGT GGC V P S R F S G AGT GAC CTG CAG CCT GAG GAT S D L Q P E D	ATG ACC CAG TCT CCA TCC TCC CTG M T Q S P S S L ACC TGC CGG GCA AGT CAG AGT ATC T C R A S Q S I 117 126 135 CCA GGA AAA GCC CCT AAA CTC CTC P G K A P K L L 171 180 189 GTC CCA TCC AGG TTC AGT GGC AGT V P S R F S G S AGT GAC CTG CAG CCT GAG GAT TTC S D L Q P E D F 279 288 297	ATG ACC CAG TCT CCA TCC TCC CTG TCT M T Q S P S S L S ACC TGC CGG GCA AGT CAG AGT ATC ATC T C R A S Q S I I 117 CDR1 - 135 CCA GGA AAA GCC CCT AAA CTC CTC ATC P G K A P K L L I 171 S R F S G S G AGT GAC CTG CAG CCT GAG GAT TTC GCA S D L Q P E D F A 279 288 297	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA M T Q S P S S L S A ACC TGC CGG GCA AGT CAG AGT ATC AGG T C R A S Q S I I R CDR1 117 CCA GGA AAA GCC CCT AAA CTC CTC ATC TTT P G K A P K L L I F GTC CCA TCC AGG TTC AGT GGC AGT GGA TCT V P S R F S G S G S AGT GAC CTG CAG CCT GAG GAT TTC GCA ACT S D L Q P E D F A T	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT M T Q S P S S L S A S ACC TGC CGG GCA AGT CAG AGT ATC ATC AGG TAT T C R A S Q S I I R Y CDR1 117 126 CCA GGA AAA GCC CCT AAA CTC CTC ATC TTT GCT P G K A P K L L I F A 171 GTC CCA TCC AGG TTC AGT GGC AGT GGA TCT GGG V P S R F S G S G S G AGT GAC CTG CAG CCT GAG GAT TTC GCA ACT TAC S D L Q P E D F A T Y 279 288 297 306	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT ATA M T Q S P S S L S A S I ACC TGC CGG GCA AGT CAG AGT ATC ATC AGG TAT TTG T C R A S Q S I I R Y L CCA GGA AAA GCC CCT AAA CTC CTC ATC TTT GCT GCA P G K A P K L L I F A A GTC CCA TCC AGG TTC AGT GGC AGT GGA TCT GGG ACA V P S R F S G S G S G T AGT GAC CTG CAG CCT GAG GAT TTC GCA ACT TAC S D L Q P E D F A T Y Y 279 288 297 306	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT ATA GGA M T Q S P S S L S A S I G ACC TGC CGG GCA AGT CAG AGT ATC ATC AGG TAT TTG AAT T C R A S Q S I I R Y L N CDR1 117 126 T CT ATA GGA CDR1 127 CCA GGA AAA GCC CCT AAA CTC CTC ATC TTT GCT GCA TCG P G K A P K L L I F A A S GTC CCA TCC AGG TTC AGT GGC AGT GGA TCT GGG ACA GAT V P S R F S G S G S G T D AGT GAC CTG CAG CCT GAG GAT TTC GCA ACT TAC TGT S D L Q P E D F A T Y Y C 279 288 297 306	ATG ACC CAG TCT CCA TCC CTG TCT GCA TCT ATA GGA GAC M T Q S P S S L S A S I G D ACC TGC CGG GCA AGT CAG AGT ATC ATC AGG TAT TTG AAT TGG T C R A S Q S I I R Y L N W 117	ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT ATA GGA GAC AGA M T Q S P S S L S A S I G D R ACC TGC CGG GCA AGT CAG AGT ATC ATC AGG TAT TTG AAT TGG TAT T C R A S Q S I I R Y L N W Y CDR1 117 CCA GGA AAA GCC CCT AAA CTC CTC ATC TTT GCT GCA TCG AAT TTG P G K A P K L L I F A A S N L CTC CCA TCC AGG TTC AGT GGC AGT GGA TCT GGG ACA GAT TTC ACT GTC CCA TCC AGG TTC AGT GGC AGT GGA TCT GGG ACA GAT TTC ACT V P S R F S G S G S G T D F T AGT GAC CTG CAG CCT GAG GAT TTC GCA ACT TAC TAC TGT CAA CAG S D L Q P E D F A T Y Y C Q Q	ATG ACC CAG TCT CCA TCC CTG TCT GCA TCT ATA GGA GAC AGA GTC M T Q S P S S L S A S I G D R V ACC TGC CGG GCA AGT CAG AGT ATC ATC AGG TAT TTG AAT TGG TAT CAG T C R A S Q S I I R Y L N W Y Q CDR1 CCA GGA AAA GCC CCT AAA CTC CTC ATC TTT GCT GCA TCG AAT TTG CAA P G K A P K L L I F A A S N L Q GTC CCA TCC AGG TTC AGT GGC AGT GGA TCT GGG ACA GAT TTC CTC V P S R F S G S G S G T D F T L AGT GAC CTG CAG CCT GAG GAT TTC GCA ACT TAC TAC TGT CAA CAG AGT S D L Q P E D F A T Y Y C Q Q S 279 288 297 306 315

Fig. 4a

LD1-110-VH sequence

		9			18									45			5
ÇAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCT	GGG	AGG	TCC	C7
	 V	 К	 L	 L	E	s	 G	- G	G	V	v	Q	P	G	R	s	
AGA	CTC	63 TCC	TGT	ATA	72 GCG	тст	GGA	81 TTC	ACC	CTC	90 AGG	AAT	TAT	99 GCC	ATG	CAC	1 T
 R	 L	s	-	 I	 А	s	 G	- 	T	L	R	N		А		Н	
GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGG	135 CTG	GAG	TGG	144 GTG	GCA		CDR1 153 ATA		TTT	1 G.
v	R	Q	A	P	G	ĸ	G	L	E	W	V	A	G	I	W - CDI	F	
GGA	AGC	17:1 AAC	AAA	AAC	TAT	GCA	GAC	189 TCC	GTG	AAG	198 GGC	CGA	TTC	207 ACC			2 A
 G		N				Α	D	S	V	К	G	R	F	T	I	S	
GAC	AAC	225 TCC	AAG		224	CTG		213		ATG	252 AAC	AGC	CTG	261 AGA		GAG	
 D	 N	 S	 К	N	Т	L		L	Н	M	N	s	L	R	Α	Ē	
ACG	GCT	279 ACA	TAT	TAC	288 TGT	GCG	AGA	297 GAG	AGG	GCG	306 ATT	CGG	GGA	315 ATC	AGT	AGA	3 T
 T	 А	т	Y	Y	C	-	R	E	R			R		I		R	
AAT	TAC	333 TAC	ATG	GAC	342 GTC	TGG	GGC	251			360			369 GTC		TCA	-
N 	•	Y CDR3		D	v	W	G	K	G	T	T	V	Т	V	s	S	

Fig. 4b

LD1-110-VL sequence

		9			18			27			36			45			54
GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC
																	т
V	M	1	Q	۵	٤	٥	3	יו	3	А	ے	٧	G	D	ĸ	V	1
		63			72			81			90			99			108
ATC	ACT	TGC	CGG	GCA	AGT	CAG	AGC	ATT	CGA	AGC	TCT	TTA	TAA	TGG	TAT	CAG	CAG
	т Т				<u></u>				 R			T.	л И	w			
-	•	Ŭ	···			_									-	ν.	-
		117	•														162
AAA	CCA	GGG															
																	S
K	P	G	r.	A	P	K	V	Ь	1	ĭ	Α,					_	
		171			180			189			198						
GGG	GTC																
G	V	P	S	R	F	S	G	R	G	S	G	T	D	F	T	Ĭ.	T
		225			234			243			252			261			270
ATC	AGC																
I	S	S	L	Q	P	E	D	F	Α	Т	Y	Y	С	Q	Q	S	S
		279			288			297			306			315			
AGT	TCC														3'		
 S	- - -	 S	 W		 F	 G	0	 G	т			 Е	I	K			
	ATC I AAA K GGG G ATC I	V M ATC ACT I T AAA CCA K P GGG GTC G V ATC AGC I S	V M T ATC ACT TGC I T C AAA CCA GGG K P G GGG GTC CCA G V P ATC AGC AGT I S 279	V M T Q ATC ACT TGC CGG I T C R AAA CCA GGG AAA K P G K GGG GTC CCA TCC G V P S ATC AGC AGT CTG I S S L	V M T Q S ATC ACT TGC CGG GCA I T C R A 117 AAA CCA GGG AAA GCC K P G K A GGG GTC CCA TCC AGG G V P S R ATC AGC AGT CTG CAG I S S L Q	GTG ATG ACC CAG TCT CCA V M T Q S P ATC ACT TGC CGG GCA AGT I T C R A S AAA CCA GGG AAA GCC CCT K P G K A P GGG GTC CCA TCC AGG TTC G V P S R F ATC AGC AGT CTG CAG CCT I S S L Q P 279 288	GTG ATG ACC CAG TCT CCA TCC V M T Q S P S ATC ACT TGC CGG GCA AGT CAG I T C R A S Q AAA CCA GGG AAA GCC CCT AAA K P G K A P K GGG GTC CCA TCC AGG TTC AGT G V P S R F S ATC AGC AGT CTG CAG CCT GAA I S S L Q P E 279 288	V M T Q S P S S ATC ACT TGC CGG GCA AGT CAG AGC I T C R A S Q S AAA CCA GGG AAA GCC CCT AAA GTC K P G K A P K V GGG GTC CCA TCC AGG TTC AGT GGC G V P S R F S G ATC AGC AGT CTG CAG CCT GAA GAT I S S L Q P E D	GTG ATG ACC CAG TCT CCA TCC TCC CTG V M T Q S P S S L ATC ACT TGC CGG GCA AGT CAG AGC AGT I T C R A S Q S I AAA CCA GGG AAA GCC CCT AAA GTC CTG K P G K A P K V L GGG GTC CCA TCC AGG TTC AGT GGC AGA G V P S R F S G R ATC AGC AGT CTG CAG CCT GAA GAT TTT I S S L Q P E D F 279 288 297	GTG ATG ACC CAG TCT CCA TCC TCC CTG TCT V M T Q S P S S L S ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA I T C R A S Q S I R CDR1 117 AAA CCA GGG AAA GCC CCT AAA GTC CTG ATC K P G K A P K V L I GGG GTC CCA TCC AGG TTC AGG GGC AGA GGA G V P S R F S G R G ATC AGC AGT CTG CAG CCT GAA GAT TTT GCG ATC AGC AGT CTG CAG CCT GAA GAT TTT GCG I S S L Q P E D F A	GTG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA V M T Q S P S S L S A ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC I T C R A S Q S I R S AAA CCA GGG AAA GCC CCT AAA GTC CTG ATC TAT K P G K A P K V L I Y GGG GTC CCA TCC AGG TTC AGT GGC AGA GGA TCT G V P S R F S G R G S ATC AGC AGT CTG CAG CCT </td <td>GTG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT V M T Q S P S S L S A S ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC TCT I T C R A S Q S I R S S L T T C R A S Q S I R S S AAA CCA GGG AAA GCC CCT AAA GTC CTG ATC TAT GCT K P G K A P K V L I Y A GGG GTC CCA TCC AGG TTC AGT GGC AGA GGA TCT GGG G V P S R F S G R G S G ATC AGC AGT CTG CAG CCT GAA GAT TTT GCG ACT TAT I S S L Q P E D F A T Y 279 288 297 306</td> <td>GTG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA V M T Q S P S S L S A S V ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC TCT TTA I T C R A S Q S I R S S L AAA CCA GGG AAA GCC CCT AAA GTC CTG ATC TAT GCA K P G K A P K V L I Y A A GGG GTC CCA TCC AGG TTC AGT GGC AGA AGA TCT GGG ACA GGG GTC CCA TCC</td> <td>GTG ATG ACC CAG TCT CCA TCC CTG TCT GCA TCT GTA GGA V M T Q S P S S L S A S V G ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC TCT TTA AAT I T C R A S Q S I R S S L N AAA CCA GGG AAA GCC CCT AAA GTC CTG ATC TAT GCT GCA TCC K P G K A P K V L I Y A A S GGG GTC CCA TCC AGS TTC AGT GGC AGA GGA TCT GGG ACA GAT G V P S R F S G R G S G T D ATC AGC AGT CTG CAG CCT GAA GAT TTT GCG ACT TAT TAT TGT I S S L Q P E D F A T Y Y C 279 288 297 306</td> <td>GTG ATG ACC CAG TCT CCA TCC CTG TCT GCA TCT GTA GGA GAC V M T Q S P S S L S A S V G D ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC TCT TTA AAT TGG I T C R A S Q S I R S S L N W CDR1 117 AAA CCA GGG AAA GCC CCT AAA GTC CTG ATC TAT GCT GCA TCC AGT K P G K A P K V L I Y A A S S GGG GTC CCA TCC AGG TTC AGT GGC AGA GGA TCT GGG ACA GAT TTC G V P S R F S G R G S G T D F ATC AGC AGT CTG CAG CCT GAA GAT TTT GCG ACT TAT TAT TGT CAA I S S L Q P E D F A T Y Y C Q 279 288 297 306</td> <td>GTG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GGA GGA GGA AGA V M T Q S P S S L S A S V G D R ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC TCT TTA AAT TGG TAT I T C R A S Q S I R S S L N W Y AAA CCA GCG CCT AAA GTC CTG ATC TAT GCT GCA TCC AGT TTG K P G K A P K V L I Y A A S S L CDR2 CDR2 CDR2<!--</td--><td>GTG ATG ACC CAG TCT CCA TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC V M T Q S P S S L S A S V G D R V ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC TCT TTA AAT TGG TAT CAG I T C R A S Q S I R S S L N W Y Q AAA CCA GGG AAA GCC CCT AAA GTC CTG ATC TAT GCA TCC AGT TTG CAA K P G K A P K V L I Y A A S</td></td>	GTG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT V M T Q S P S S L S A S ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC TCT I T C R A S Q S I R S S L T T C R A S Q S I R S S AAA CCA GGG AAA GCC CCT AAA GTC CTG ATC TAT GCT K P G K A P K V L I Y A GGG GTC CCA TCC AGG TTC AGT GGC AGA GGA TCT GGG G V P S R F S G R G S G ATC AGC AGT CTG CAG CCT GAA GAT TTT GCG ACT TAT I S S L Q P E D F A T Y 279 288 297 306	GTG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA V M T Q S P S S L S A S V ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC TCT TTA I T C R A S Q S I R S S L AAA CCA GGG AAA GCC CCT AAA GTC CTG ATC TAT GCA K P G K A P K V L I Y A A GGG GTC CCA TCC AGG TTC AGT GGC AGA AGA TCT GGG ACA GGG GTC CCA TCC	GTG ATG ACC CAG TCT CCA TCC CTG TCT GCA TCT GTA GGA V M T Q S P S S L S A S V G ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC TCT TTA AAT I T C R A S Q S I R S S L N AAA CCA GGG AAA GCC CCT AAA GTC CTG ATC TAT GCT GCA TCC K P G K A P K V L I Y A A S GGG GTC CCA TCC AGS TTC AGT GGC AGA GGA TCT GGG ACA GAT G V P S R F S G R G S G T D ATC AGC AGT CTG CAG CCT GAA GAT TTT GCG ACT TAT TAT TGT I S S L Q P E D F A T Y Y C 279 288 297 306	GTG ATG ACC CAG TCT CCA TCC CTG TCT GCA TCT GTA GGA GAC V M T Q S P S S L S A S V G D ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC TCT TTA AAT TGG I T C R A S Q S I R S S L N W CDR1 117 AAA CCA GGG AAA GCC CCT AAA GTC CTG ATC TAT GCT GCA TCC AGT K P G K A P K V L I Y A A S S GGG GTC CCA TCC AGG TTC AGT GGC AGA GGA TCT GGG ACA GAT TTC G V P S R F S G R G S G T D F ATC AGC AGT CTG CAG CCT GAA GAT TTT GCG ACT TAT TAT TGT CAA I S S L Q P E D F A T Y Y C Q 279 288 297 306	GTG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GGA GGA GGA AGA V M T Q S P S S L S A S V G D R ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC TCT TTA AAT TGG TAT I T C R A S Q S I R S S L N W Y AAA CCA GCG CCT AAA GTC CTG ATC TAT GCT GCA TCC AGT TTG K P G K A P K V L I Y A A S S L CDR2 CDR2 CDR2 </td <td>GTG ATG ACC CAG TCT CCA TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC V M T Q S P S S L S A S V G D R V ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC TCT TTA AAT TGG TAT CAG I T C R A S Q S I R S S L N W Y Q AAA CCA GGG AAA GCC CCT AAA GTC CTG ATC TAT GCA TCC AGT TTG CAA K P G K A P K V L I Y A A S</td>	GTG ATG ACC CAG TCT CCA TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC V M T Q S P S S L S A S V G D R V ATC ACT TGC CGG GCA AGT CAG AGC ATT CGA AGC TCT TTA AAT TGG TAT CAG I T C R A S Q S I R S S L N W Y Q AAA CCA GGG AAA GCC CCT AAA GTC CTG ATC TAT GCA TCC AGT TTG CAA K P G K A P K V L I Y A A S

Fig. 5a

LD1-117-VH sequence

		9			18			27		C TO C	36	C N C	CCT	45		TCC	
CAG	GTG	AAA	CTG	CTC	GAG	TCA	GGA	GGA							AAG		
Q	V	K	L	L	E	S	G	G	G	V	V	Q	P	G	K	S	L
		63			72			81			90			99			108
AGA	CTT	TCC	TGT	GCA	GCG	TCT	GGA	TTC	AGT	TTC	AAT	AGC	CAT	GGC	ATG	CAC	TGG
 R	 L	 S	c	 A	A	s	G	F	S	F	И	s	Н	G	М	H	W
		117			126			135			144	ccn	en crear	153	TICC.	மன்க	162 GAT
GTC	CGC	CAG	GCT	CCA	GGC -	AAG	GGG	CTG	GAG	TGG	GT.G	GCA			TGG		
	 R	Q						L			V	Α	F	I	W	F	D
													4		CDR2		
		171			180			189			198		mmo	207	n mc	N.C.C	216
GGC	AGT	AAT	AAA	TAC	TAT	GCA	GAC	TCC	GTG	AAG	GGC	CGA	110	ACC	ATC	ACC	
- - -	5	N	K	Y	Y	A	D	s	V	ĸ					I		R
					CI	DR2 -								261			270
		225			234			243	~ N N	א ידו כ־	252		כידים				
GAC	AAC	TCC			ACG		TAT	CTG	CAA	AIG					GCC		
ם	и	s	K					L			N	S	L	R	Α	E	D
		220			200			297			306			315			324
ACG.	сст	Z / S GTC	тат	TAC	TGT	GCG	AGA	GAG	ACC	TCA	GTA	AGG	CTA	GGG	TAT	AGC	CGC
T	А	V	Y	Y	С	Α									Y		R
											260	— CD	R3 —	260			378
m » c	2.20	333	TT N.C	אתכ	342	GTC	TGG	351 GGC	ααα	GGG	ACC	ACG	GTC	ACC	ATC	TCG	
1 AC	AAT													- 			
Y	N	Y	Y	M	D	V	W	G	K	G	T	T	V	Т	I	S	S
			- CDR	3			•										

Fig. 5b

LD1-117-VL sequence

		9			18			27			36			45			54
GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC
v	M	T	Q	s	P	S	s	L	S	A	s	V	G	D	R	V	T
		63												99			
ATC	ACT	TGC	CGG	GCA	AGT	CAG	AGC	ATT	AGG	AGC	CAT	TTG	AAT	TGG	TAT	CAG	CAG
I	T	С	R	А		-			R					W	Y	Q	Q
								— CI)R1 -								1.60
מממ	CCD	117	אאא	ccc	126	ת א כ	CTC	135	אתכ	ሞልሞ	144 GCT	GCA	TCC	153 AGT	ттс	CAA	GGT
ĸ	P	G	К	A	P	К	L	L	I	Y	А	А	s	S	L	Q	G
														CDR2			
		171			180			189			198		C 1 T		x cm	cmc	216
GGG	GTC	CCA	TCA	AGG	TTC	AGT	GGC	AGT	GGA	TCT		ACA	GAT	TTC	ACI		ACC
G	V	P	S	R	F	\$	G	S	G	S	G	T	D	F	T	L	T
		225			234			243			252			261			270
ATC	AGC	AGT	CTG	CAA	CCT	GAA	GAT	TTT	GCA	ACT	TAT	TAC	TGT	CAA	CAG	AGT	TAC
	s	s	 L	Q	P	 E	D	 F	Α	T	Υ	 Y	c	Q	Q	s	Y
		270			200			207			206			← 315			
AGG	GCC		CAG											ATC		3'	
 R	 А	P	 Q	w	 T	 F	 G	Q	 G	T	К	V	E		K		
		. CDP	3														

Fig. 6a

LD2-1-VH sequence

		9			18			27			36			45			54
CAG	GТG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCG	GGG	GGG	TCC	CTG
 Ω	 V	K	L	L	Ē	s	G	G	G	v	V	Q	P	G	G	S	L
		63			72			81			90						
AGA	CTC	TCC	TGT	GTA	GCG	TCT	GGA	TTC	ACC	CTC	AGG	AGT	TAT	GGC	ATG	CAC	TGG
 R	 L	 S			 А	 S	 G	F	T	L	R	s	Y	G	М	Н	W
• •	_													CDR1			→
		117			126			135		mcc.	144	~ ~ ~	T T T	153	TGG		
GTC	CGC	CAG	GCT	CCA	GGC	AAG	GGC	CTG		766	GTG						
v	R	Q	A	P	G	K	G	L	Ε	W	V	Α	F	I	W	-	D
		-													CDR2		016
		171			180	cm.	~~~	189	cm.c	220	198	CCA	ምጥር	207	ΔTC	TCC	216 CGA
GGA	AGT	AAT	AAA	GGA	TAT	GTA	GAC	100	GTG	AAG	GGC						
G	S	И	к	G	Y	V	D	S	V	K	G	R	F	T	I	S	R
						R2 —											270
		225	226	220	234	CTC.	T > T	243	C D D	አጥር	252 AAC	AGC	CTG		GCC		
GAC	AAT	700	AAG		AIG												
D	И	S	K	N	М	V	Y	L	Q	М	N	S	L	R	A	Đ	D
		279			288			297			306			315			324
ACG	GCT	GTA	TAT	TAT	TGT	GCG	AGA	GAG	AAG	GCG	CTT	CGG	GGA	ATC	AGC	AGA	
 T	 A	 V	 Y	 Y		 А	R	 Е	K	А	L	R	G	Ι	s	R	Y
												- CD	R3 —	260			
ם מ כ	тΔт	333 TAC	ርጥር	GAC	342 GTC	TGG	GGC	351 AAG	GGG	ACC	360 ACG	GTC	ACC	GTC	TCC	TCA	3'
								-									
N	Y	Y	L	D	V	W	G	K	G	T	T	V	Т	V	S	S	
		CDR3															

Fig. 6b

LD2-1-VL sequence

GTG		9	a. c	000	18	mc N	ccc	27	ccc	۸۲۲			CAG		GTC	ACC	54 ATC
GTG	GTG	ACT	CAG	CCA		TCA											
v	v	T	Q	P	P	S	A	s	G	T	₽	G	Q	R	V	T	I
		63			72						90						108
TCT	TGT	TCT	GGA	AGC	AAC	TCC	ATC	CTT	GGA	AGT	AAG	TAT	GTA	TAC	TGG	TAC	CA
s		S	- G	3			I	L	G	s	К	Y		Y	W	Y	Q
							c	DR1			1 4 4			153			16
AAA	СТС	117 CCA	GGA	ACG	GCC	ссс	AAA	CTC	CTC	ATC	144 TAT	AAG	AAT	GAT	CAG	CGG	
 K	 L	 P	- <i></i>		Α	P	K	L L	 L	I		К		D		R	P
																	
		171			180			189			198		200	207		TCC	
TCA	GGG	GTC	TC T	GAC	CGA	TTC	TCT	GGC	TCC	AAG	TCT	GGC	ACC	TCG			
 s	G		s	5	R	F	s	G	S	K	S	G	T	S	А	S	L
$\stackrel{\longrightarrow}{-\!\!\!\!-\!\!\!\!-\!\!\!\!-}$					224			242			252			261			27
GCC	ATC	225 AGT	GGG	CTC	234 CGG	TCC	GAG	GAT	GAG	GCT	GAC	TAT	TAC	TGT		CCA	TG
- - -	I	- - -	G	 1	 R	s	 E	D	E	A	D	Y	Y	С	A	P	W
								207			206			715	-		32
GAT	GCC	279 AA C	CTG	GGT	GGC	CCG	GTG	TTC	GGC	GGA	GGG	ACC	AAG	CTG			
D			 L						G	G	G	т	К	L	T	V	L
				DR3 -				→									
	CAG																

BNSDOCID: <WO__9749809A1_I_>

Fig. 7a

LD2-4-VH sequence

CAG	GTG	9 AAA	CTG	CTC	18 GAG	TCG	GGG	GGA	GGC	GTG							
Q	 V		L	L L	E	S	G						Р	G	G	S	L
AGA	CTC	63 TCC	TGT	GAA	72 GCG	TCT	GGA	TTC	ACC	CTC	AGA	AGT	TCT	99 GGC	ATG	CAC	100 TG
R		s		E	A	s	G	£	т					G CDR1	М	H	W
GTC	CGC	117 CAG	GCT	CCT	126 GGC	AAG	GGG	135 CTG	GAG	TGG	GTG		CTT	153			16
V	 R	Q	 А	p P	G	 К	G	L	E			Α	L	I	W	F	D
GGA	AGT	171 ATC	AGA	TCG	180 TAT	GCA	GAA	189 TCC	GTG	AAG 	198 GGC			207 ACC			21
G	S	I	3				E			K	G	R	F	T	I	S	R
GAC	АСТ	225 TCC	AAG		234		TAT	243		ATG	CGC	AGT	CTG			GAC	
	 Т	 S	K	и 	т		 Y	L	Q	M	 R	s	L	S	A	D	D
ACG	GCT	279 GT G	TAT	TAC	288 TGT	GCG	AGA	GAC	AAG	GCG	GTT	cgg	GGA		AGC		32 ·
 T	-	v	Y	Υ	C	A		D	K	A	V	R			S	R	Y
AAC	TAT	333 TAC		GAC	342 GTC	TGG	GGC	351			360	GTC		369		TCA	3
N	•	Y - CDI	 М R3 —			~ ₩ →	G	K	G	T	Т	V	Т	V	5	S	

Fig. 7b

LD2-4-VL sequence

		9			18			27			36			45			54
GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC
v	M	T	Q	5	P	S	s	L	S	A	S	v	G	D	R	V	Т
ATC	ACT	63 TGC	CGG	ACA	72 AGT	CAG	ACC	81 T TA	AGC	AGA	90 AAT	TTA	AAT	99 TGG	TAT	CAG	108 CAG
 I		 C		T	 S	Q	 T	 I	 S	 R	N	 L				Q	Q
AAA	CCA	117 GGG	AAA	GCC	126			135			144 GCT		TCC		TTG		
к	P	G G	 К	Α	P	 К	L	L	I	Y		T	5	S CDR2		~	
GGG	GTC	171 CCA	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GGA	TCT	198 GGG	ACA		207			216 AC
G	V	Р	-	R	F	s	G	s	G ′	S	G	T	D	F	Т	L	Т
ATC	AAT	225 AGT	CTA	CAA	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG		270 TAG
I	N	s	L	Q	P	E	D	- F	Α	T	Y	Y	С	Q	Q	S	Y
ACT	ACC	279 CCT	TCG	TTC	288 GGC	CAA	GGG	297 ACC	AAG	GTG	306 GAA	ATC	AAA	315 3'			
T	-	P R3 —	 \$ →	F	G	Q	G	Т	K	V	E	ī	K				

Fig. 8a

LD2-5-VH sequence

			9			18			27		mm .c	36	CAC	ccc	45	ccc	ጥርር	54 CTG
5'	CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	TTG	GTC						
	Q		- 	L	L	E	S	G	G	G	L	V	Q	P	G	G	S	L
	AGA	CTC	63 TCC	TGT	GTA	72 GCG	TCT	GGA	81 TTC	ACC	TTC	90 AGG	AGT	TAT	99 GGC 	ATG	CAC	108 TGG
	 R	 L	 S		v	,A	 S	G	F	T	F	R		Y		_	Н	W
	GTC	CGC	117 CAG	GCT	CCA		AAG	GGC	CTG		TGG	GTG 	GCT	TTT 	ATA 	TGG	TTT	
	V	R	Q	А	P	G	K	G	L	E	W	V				CDD3	F	D
	GGA	AGT	171 AAT	AAA 	GGA	TAT	GTA		TCC		AAG 	GGC	CGA	TTC	207 ACC	ATC	TCC	216 CGA
	G	S	N	K	G					V	K	G .	R	F	Т	Τ	S	r
	GAC	AAT	225 TCC		AAC	224			243		ATG	252 AAT	AGC	C T G	261 AGA		GAG	270 GAC
		 N	s		N N	 М	L	Y		Q	М	N	S	L	R	A	Ε	D
	ACG	GCT	279 GTA	TAT	TAT	288 TGT	GCG	AGA	297 GAG	AAG	GCG	306 CTT	CGG	GGA	315 ATC	AGT	AGA	324 TAC
	 T	 A		 Y	Y	C		R	F	К	А	L	R	G		S		Y
	AAC	TAT	333 TAC	CTG	GAC	342 GTC	TGG		351 AAG 		GCC	360 ACG	GTC	ACC	369 GTC	TCC	TCA	3'
	И	Y	_	D 3 L	-	V	W	G	K	G	A	Т	V	T	V	5	۵	

Fig. 8b

LD2-5-VL sequence

GTG	አጥር	9	CAG	ጥርጥ	18 CCA	TCC	TCC	27 CTG	TCT	GCA	36 TCT	ATA	GGC	45 GAC	AGA	GTC	54 ACC
 V	 M	 T	 Q	 S	 P	-	 S		 S		 S		 G		 R	 V	 T
·		63 TGC	CGG	GCA	72 AGT	CAG	AGC	81 GTT	ACC	AGG	90 TCT	TTA	AAT	99 TGG	TAT		108 CAG
 I	 T	 C	 R	 A		Q	s	v		R	s	L		M 	Y		Q
AAA	CCA	117 GGG	AAA	GCC	126 CCT			135		TTT	144		тсс 	153 ACT	TTG	CAA	162 AGT
	P	G	K	Α	P	R	L	L	I	Ē	Α	A		Т		Q	s
GGG	GTC	171 CCA	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GGA	TCT	198 GGG	ACA		207			216
G		P	S	R	F	s	G	S	G	s	G	Т	D	F	Т	L	T.
ATC	AGC	225 AGT	CTG	CAA	234 CCT	GAG	GAT	243 TTT	GGA	ACT	252 TAC	TAC	TGT	261 CAA		AAT	270 TAC
	- - -	 S	 L	Q	P	E	D	F	G	T	Y	Y	С	Q	Q	Ν	Y
AGG	ACC	279 CCT	CAG	TGG	288 ACG	TTC	GGC	297 CAA	GGG	ACC	306 AA G	GTA	GAA	315 ATC	AAA	. 3'	
P.	 T	P	Q :DR3	w ———	т		-	Q	G	T	ĸ	V	E	*	K		

Fig. 9a

LD2-10-VH sequence

CAG	GTG	9 AAA	CTG	СТС	18 GAG	TCT	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCG	45 GGG	GGG	TCC	54 CTG
	 V			 L	 E	 S	-	 G		v			P	G	G	S	L
_				GTA	72 GCG	TCT	GGA	81 TTC	ACC	CTC	90 AGG	AGT	TAT	99 GGC	ATG	CAC	108 TGG
 R					 А			 F		L	R	S	Y	G	M	H	W
GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGC	135 CTG	GAG	тgg	144 GTG	GCT		153			162
	 R	 Q			 G						_	A	F	I	W	F	D
GGA	AGT	171 AAT	AAA	GGA	TAT	GTA	GAC	189 TCC	GTG	AAG	198 GGC	CGA		207	CDR2 ATC		216 CGA
	 S	_ ~ -		-	Y	V		s			G		F		I	S	R
GAC	AAT	225 TCC	AAG		- CDI 234 ATG		TAT	243 CTG	CAA	ATG	252 AAC		CTG			GAT	270 GAC
- D	 N	 S	 K	N	 М	v	Y	L	Q	М		s	L	R	А	D	D
ACG	GCT	279 G T A	TAT	TAT	288 TAT	TGT	GCG	297 AGA	GAG	aag	306 GCG	CTT	CGG	315 GGA	ATC	AGC	324 AGA
 T	 A	 V	Y		 Y		 A		E	К	А	L	R	G	I		
TAC	. AAC	333 TAT	B TAC	стс	342 GAC	GTC	TGG	351 GGC	•		360	ACG		369		TCC	378 TCA
 Y	N	Y	Y	L	D	v	W	G	К	G	T	T	V	T	V	S	S

_____ CDR3 —

Fig. 9b

LD2-10-VL sequence

		9			18			27			36			45			
GTG	GTG	ACT	CAG	GAG	CCC	TCA	CTG	ACT	GTG	TCC	CCA	GGA	GGG	ACA	GTC	ACT	CTC
v	V	т	Q	- <u>-</u> -	P	s	L	T	V	s	P	G	G	T	V	T	L
ACC	TGT	63 GCT	TCC	AGC	72 ACT	GGG	GCA	81 GTC	ACC	AGG	90 GGT	TAC	TAT	99 CCA	AAC	TGG	108 TTC
 T	 C	Α	S	S	 Т		Α							P	N	W	F
CAG	CAG	↓17 AAG	ССТ	GGA	126		ccc	135			144				AAC	AAA	162 AAA
Q	Q	 К	P	G	Q	Α	P	R	A	L	I	Y	s —	T	N CDR	к 2 —	К
CAC	TCC	171 TGG	ACC	сст	180 GCC	CGG	TTC	189 TCA	GGC	TCC	198 CTC	CTT	GGG	207 GGC	AAA	GCT	216 GCC
н	s	w	T.	P	Α	R	F	S	-	s	L	L	G	G	K	A	Α
CTG	→ ACA	225 CTG	TCA	GGT	234 GTG	CAG	CCT	243 GAA	GAC	GAG	252 GCT	GAA	TAT	261 TAC		CTG	270 CTC
L	т	L	S	G	V	Q	₽	Ε	D	Ε	Α	E	Y	Y	С	L	L
TAC	TAT	279 GGT	GGT	GCT	288 CAA	CTC	GTA	297 TTC	GGC	GGA	306 GGG	ACC	AAG	315 CTG		GTC	324 CTA
-	Y	_	G	Α	Q		V	F	G	G	G	Т	K	I.	Т	V	L
		333 CCC		(3													

Fig. 10a

LD2-11-VH sequence

		9			18									45			54
CAG	GTG	AAA	CTG	CTC	GAG	TCG	GGG	GGA	GGC	GTG	GTC	CAG	CCG	GGG	GGG	TCC	CTG
Q	V	 К	L	L	E	s	G	G	G	ν	V	Q	₽	G	G	S	L
AGA	стс	63 TCC	TGT	GAA	72 GCG	TCT	GGA	81 TTC		CTC	90 AGA			99 GGC	ATG	CAC	108 TGG
R	L	S	С	E	A	3	G	F	Т	L	R	S	S		M	• • •	W
GTC	CGC	117 CAG	GCT	CCT	126 GGC	AAG	GGG	135 CTG	GAG	TGG	144 GTG			153	TGG	TTT	162 GAT
V	R	Q	А	P	G	K	G	L	Е	W	V	А	L	I	W CDR2	_	D
GGA	AGT	171 ATC	AGA	TCG	180 TAT	GCA	GAA	189 TCC	GTG	AAG	198 GGC	CGA	TTC	207			
-	 S		 R	s	Y	A	E	3	V	К	G	R	·F	T	ı	S	R
GAC	ACT	225 TCC	AAG		234	R2 — CTA		243 CTC	CAA	ATG	252 CGC	AGT	CTG	261 AGT	GCC	GAC	270 GAC
D	 T	s		N	T	L	Y	L	Q	M	R	S	L	S	Α	D	D
ACG	GCT	279 GTG	TAT	TAC	288 TGT	GCG	AGA	297 GAC	AAG	GCG	306 GTT	CGG	GGA	315 ATT		AGG	324 TAC
т	 А	V	Y	Ϋ́	C	A	R	D	K		V	R	G			R	Y
AAC	TAT	333 TAC	ATG	GAC	342 GTC	TGG	GGC	351 AAA			360 ACG		-	369		TCA	3 '
N	Y	<u>-</u> Y	 М	D	V	w	G	K	G	T	T	V	T	V	S	S	
		— CD	R3 —														

Fig. 10b

LD2-11-VL sequence

		9			18			27			36		223	45	202	CT C	54
GTG	TTG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	ATA	CGA	GAC			
v	L	T	Q	s	Þ	S	s	L	S	Α	S	I	R	D	R	V	Т
		63			72			81	0.00	7 CT	90			99 TGG		CAG	108 CAC
ATC	ACT	TGC	CGG	GCA	AGT	CAG	AAC	ATT		AGI			- - -				
	T	С	R	А			N					L	И	W	Y	Q	Н
		117			126			135			144		 →	153		~ N N	162
AAA	CCA	GGG	ACA	GCC	CCT	AAA	CTC	CTG	ATC	TAT	GCT	GTA	TCC	GCT			AG.
 K	 P	 G	- - -	 А	P	K	L	L	I	Y	А			A		Q	S
				•							100						21
GGG	GTC	171 CCA	TCG	AGG	180 TTC	AGT	GGC	189 AGT	AGA	TCT	GGG	ACA	GAT			CTC	
 G		 P	s	R	F	s	G	s	R	S	G	Т	D	F	T	L	Т
ATC	AGC	225 AGT	CTG	CAA	234 CCT	GA.A	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA		AGT	27 TA
 I	- - -		 L	 Q	 P	 E		F		T			C			S	Y
		279			288			297			306			315			
AGT	CCC	CCG	TAC	ACT	TTC	GG C	CAG	GGG	ACC	: AAC	CTG	CAG	ATC	. AAA	3'		
 S	-	_	Y		F	- G	Q	 G	T	N	L	Q	I	K			

Fig. 11a

LD2-14-VH sequence

GGG G	TCC	CTG
G	S	т
		Ţ
		108
ATG	CAC	TGG
М	Н	W
· —		
ፕሮር	ம்ம்ம	162 GAT
M	F	D
CDR2		216
GTC	TCC	
V	S	R
Α	E	D
		324
TCC	TCA	. 3'
-	_	
	TGG W CDR2 GTC V GCC A AGT S	M H TGG TTT W F CDR2 GTC TCC V S GCC GAA A E AGT AGA S R

Fig. 11b

LD2-14-VL sequence

		9			18			27			36			45			54
GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTG	GGA	GAC	AGA	GTC	ACC
 V	 M	 Т	Q	- 	P	s	s		s	A	s	V	G	D	R	V	T
ATC	ACT	63 TGC	CGG	GCA	72 AGT	CAG	AGC	81 ATT	ATC	AAC	0 Q TAA	ATT	AAT	99 TGG	TAT	CAG	108 CAC
 I	 T		 R	 A	-	Q	s		I	N	И	L					
AAA	CCA	117 GGC	← AAA		300			135 CTG	ATC	TAT	144			153 AGT			163 AG
			 K								A	A	S	S	L	Q	3
GGG	GTC	171 CCT	TCA	AGG	TTC	CGT	GGC	AGT	GGA	TCT	198 GGG	AGA	GAT	TTC	ACT	CTC	21 AC
- - -		P	s	R	F	R	G	s	G	s	G	R	D	F	Т	L	Т
GTC	ACC	225 AGT	CTG	CAA	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG		
	 T	- S	- L	Q	 P	 E	D	F	A	T	Y	Y	С	Q	Q	S	Υ
AG T	ACC	279 CTG	TGG	ACG	288 TTC	GGC	CAA	297 . GGG	ACC	AAG	306 GTG	GAA	ATC	315 AAA			
 S	 Т	 L	 W	 T	 F	 G	Q	- -	T	К	V	E	I	K			

Fig. 12a

LD2-17-VH sequence

CAG	GTG	9 AAA	CTG	CTC	18 GAG	тст	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCG	45 GGG	GGG	TCC	C'
			 L		 E					V	V	Q	P	G	_	s	
_					72 GCG	TCT	GGA	TTC	ACC	TTC	90 AGG	AGT	TAT	99 GGC	ATG	CAC	1 (T)
		 s			Α			 F		F	R	S	Y	G CDR1	M	H →	
GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGC	CTG	GAG	TGG	GTG	`		153		TTT	1 G.
 V	 R	 Q	 A								V		F	I	W	F	
GGA	AGT	171 AAT	AAA	GGA	180 TAT	GTA	GAC	TCC	GTG	AAG	198 GGC	CGA		207			2
- - - G			~ К	G	Y	V	D	s	ν	K	G	R	F	Т	I		
GAC	AAT	225 TCC					TAT			ATG	252 AA G	AGC	CTG	261 AGA	GCC	GAG	2 5 G
	N 	 S	 K				Y				K	S	L	R	Α	E	
ACG	GCT	279 GTA	IAT	TAT	288 TGT	GCG	, AGA	GAG	, AAG	GCG	306 CTT	CGG	GGA	315 ATC		AGA	
	 A					A	R	E	к	 A	L	R	G	I	S	R	
_			3 C CTC	G GAG	342 C GTC	: TGC	G GGC		G GGC	ACC	360 ACG	CDR1		369)	TC#	٦. -
 N		Y		D		w	G	К	G	Т	Т	V	Т	V	S	S	

Fig. 12b

LD2-17-VL sequence

5'	GTG	ATG	9 ACC	CAG	TCT	18 CCA	TTC	TCC	27 CTG	TCT	GCA	36 TCT	GTA	GGA	45 GAC	AGA	GTC	54 ACC
	 V	 М	 Т	Q	 s	P	F	S	L	\$	A	s	V	G	D	R	V	Т
	ATC	ACT	63 TGC	CGG	GCA	72 AGT	CAG	AAC	81 ATT	AGG	AGT	90 TTT	TTA			TAT		108 CAG
		 Т	 С	 R	 A	5		N 						S	W	Y	Q	Q
	AAA	CCA	117 GGG	← ACA	GCC	CCT	AAG		135 CTG	ATC	TAT	144 GCT	GCA	TCC	AGG		CAA	162 AGT
	ĸ	P	G G	T	À	P	K	L	L	I	Y	Α	A	S	R	L	Q	S
	GGG	GTC	171 CCA	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GGG	TCT	198			207			216
	G	V	P	S	R	F	S	G	S	G	s	G	T	D	F	Т	L	Т
	ATC	AGC	225 ACT	CTG	CAA	234 CCT	GAA	GAT	243 TTT	GCG	ACT	252 TAC	TAC	TGT	261 CAA		AGT	
		 S	 T	 L	Q	P P	- - -	D	F	A	Т	Y	Y	С	Q	Q	S	Y
	AGT	. ecc	279	TGG	ACG	288 TTC	GGC	CAA	297 . GGG	ACC	AAG	306 CTG	GAA	ATC	315 AAA			
	_		P CDR3	w ——		F	G	Q	G	Т	K	L	£	I	К			

Fig. 13a

LD2-20-VH sequence

GTG	9 AAA	c T G	CTC	18 GAG	TCT	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCG	45 GGG	GGG		54 CTG
		 L		 E	s	 G	G G	G			Q	P	G	G	S	L
			GTA	72 GCG	тст	GGA	81 TTC	ACC	TCC	90 AGG	AGT	TAT	99 GGC	ATG	CAC	108 TGG
			 V	 A	 s	 G	 F				S	Y	G	M	Н	W
			CCA	126 GGC	AAG	GGC	135 CTG	GAG	TGG	144 GTG	`		153		TTT	162 GAT
 R	 Q	 А	P	 G					W			F	1	W	F	D
AGT	171 AAT	AAA	GGA	180 TAT	GTA	GAC	189 TCC	GTG	AAG	198 GGC	CGA		207			216
		- - -	 G											I	S	R
							243 CTG	CAA	ATG	252 AAG	AGC	CTG	261 AGA	GCC	GAG	270 GAC
				 T	 L	Υ	L	Q	М			L	R	Α	E	D
		TAT	TAT	288 TGT	GCG	AGA	297 GAG	AAG	GCG	306 CTT	CGG	GGA	315 ATC	AGT	AGA	324 TAC
					A	R			А	L	R	G	I	S	R	Y
		B CTC	GAC	342 C GTC	? TGG	GGC	351 AAC	G GGG		260			369	TCC	TC#	4 3 ' -
. -		 L													S	
	CTC L CGC R AGT N GCT A	V K 63 CTC TCC L S 117 CGC CAG R Q 171 AGT AAT S N 225 AAT TCC N S GCT GTA A V 333 TAT TAC	V K L 63 CTC TCC TGT L S C 117 CGC CAG GCT R Q A 171 AGT AAT AAA S N K 225 AAT TCC AAG N S K 279 GCT GTA TAT A V Y 3333 TAT TAC CTC	V K L L 63 CTC TCC TGT GTA L S C V CGC CAG GCT CCA R Q A P 171 AGT AAT AAA GGA S N K G 225 AAT TCC AAG AAC N S K N GCT GTA TAT TAT A V Y Y TAT TAC CTG GAC	GTG AAA CTG CTC GAG V K L L E 63 CTC TCC TGT GTA GCG L S C V A 117 CGC CAG GCT CCA GGC R Q A P G 171 AGT AAT AAA GGA TAT S N K G Y CDI 225 AAT TCC AAG AAC ACG N S K N T 279 GCT GTA TAT TAT TGT A V Y Y C 3333 TAT TAC CTG GAC GTC	GTG AAA CTG CTC GAG TCT V K L L E S 63	GTG AAA CTG CTC GAG TCT GGG V K L L E S G CTC TCC TGT GTA GCG TCT GGA L S C V A S G CGC CAG GCT CCA GGC AAG GGC R Q A P G K G AGT AAT AAA GGA TAT GTA GAC S N K G Y V D CDR2 AAT TCC AAG AAC ACG CTC TAT N S K N T L Y GCT GTA TAT TAT TGT GCG AGA A V Y Y C A R 333 TAT TAC CTG GAC GTC TGG GGC	GTG AAA CTG CTC GAG TCT GGG GGA V K L L E S G G CTC TCC TGT GTA GCG TCT GGA TTC L S C V A S G F CGC CAG GCT CCA GGC AAG GGC CTG R Q A P G K G L 171 AAA GGA TAT GTA GAC TCC S N K G Y V D S CDR2 225 AAT TCC AAG AAC ACG CTC TAT CTG N S K N T L Y L GCT GTA TAT TAT TGT GCG AGA GAG A V Y Y C A R E 333 342 351 TAT TAC CTG GAC GTC TGG GGC AAG	GTG AAA CTG CTC GAG TCT GGG GGA GGC V K L L E S G G G CTC TCC TGT GTA GCG TCT GGA TTC ACC L S C V A S G F T CGC CAG GCT CCA GGC AAG GGC CTG GAG R Q A P G K G L E AGT AAT AAA GGA TAT GTA GAC TCC GTG S N K G Y V D S V CDR2 AAT TCC AAG AAC ACG CTC TAT CTG CAA N S K N T L Y L Q GCT GTA TAT TAT TGT GCG AGA GAG AAG A V Y Y C A R E K 333 342 351 TAT TAC CTG GAC GTC TGG GGC AAG GGC A A G GC A A G GC	GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG V K L L E S G G G V 63 CTC TCC TGT GTA GCG TCT GGA TTC ACC TCC L S C V A S G F T S CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG R Q A P G K G L E W AGT AAT AAA GGA TAT GTA GAC TCC GTG AAG S N K G Y V D S V K CDR2 225 AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG N S K N T L Y L Q M 279 GCT GTA TAT TAT TGT GCG AGA GAG AAG GCG A V Y Y C A R E K A 333 TAT TAC CTG GAC GTC TGG GGC AAG GGG ACC	GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC V K L L E S G G G V V 63 CTC TCC TGT GTA GCG TCT GGA TTC ACC TCC AGG L S C V A S G F T S R CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG R Q A P G K G L E W V AGT AAT AAA GGA TAT GTA GAC TCC GTG AAG GGC S N K G Y V D S V K G CDR2 225 AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG AAG N S K N T L Y L Q M K 279 GCT GTA TAT TAT TGT GCG AGA GAG AAG GCG CTT A V Y Y C A R E K A L 333 342 342 351 AAG GGG ACC ACG	9 18 GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG V K L L E S G G G V V Q 63 72 81 90 CTC TCC TGT GTA GCG TCT GGA TTC ACC TCC AGG AGT L S C V A S G F T S R S CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT R Q A P G K G L E W V A AGT AAT AAA GGA TAT GTA GAC TCC GTG AAG GGC CGA S N K G Y V D S V K G R CDR2 234 AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG AAG AGC N S K N T L Y L Q M K S CTA TAT TAT TAT TGT GCG AGA GAG GAG AAG GCG CTT CGG A V Y Y C A R E K A L R 3333 342 342 351 360 GTC TT T V	9 GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCG V K L L E S G G G V V Q P 63 CTC TCC TGT GTA GCG TCT GGA TTC ACC TCC AGG AGT TAT L S C V A S G F T S R S Y CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GTG GCT TTT R Q A P G K G L E W V A F 171 AGT AAT AAA GGA TAT GTA GAC TCC GTG AAG GGC CGA TTC S N K G Y V D S V K G R F AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG N S K N T L Y L Q M K S L 279 GCT GTA TAT TAT TGT GCG AGA GAG AAG GGG CCTT CGG GGA A V Y Y C A R E K A L R G 1333 TAT TAC CTG GAC GTC TGG GGC AAG GGG ACC ACG GTC ACC TTT T V T TAT TAC CTG GAC GTC TGG GGC AAG GGG ACC ACG GTC ACC	9	GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCG GGG GGG V K L L E S G G G V V Q P G G CTC TCC TGT GTA GCG TCT GGA TTC ACC TCC AGG AGT TAT GGC ATG L S C V A S G F T S R S Y G M CDR1 117 CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GTG GTG GTG ATG GTG ATG A	9 18 27

Fig. 13b

LD2-20-VL sequence

5 '	GTG	ATG	9 ACC	CAG	TCT	18 CCA	TCC	TCC	27 CTG	TCT	GCA	36 TCT	GTA	GGA	45 GAC	AGA	GTC	54 ACC
	V	M	T	Q	s	P	S	s	L	s	Α	s	V	G	D	R	V	T
	7 W.C	አ ርጥ	63 TGC	CGG	GCA	72 AGT	CAG	AGC	81 ATT	AGC	AGC	90 TAT	TTA	ТАА	99 TGG	TAT	CAG	108 CAG
	A1C																	
	1	T	С	R	A.	S									W	ĭ	Q	Q
	AAA	CCA	117 GGG	AAA	GCC	126 CCT			135			144	GCA		153 AGT	TTG	CAA	162 AGT
	 К	 Р	 G	 К	- 	P	ĸ	L	L	I	Y	Α	Α	_	S		-	
	GGG	GTC	171 CCA	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GGA	TCT	198			- CD 207 TTC			216
	 -	 V	 P	 S	R	F	s	G	S	G	s	G	Т	D	F	T	L	T
	ATC	AGC	225 AGT	CTG	CAA	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	270 TAC
	 I	 S	S		Q	P	Ē	D	£	Α	т	Y	Y	С	Q	Q	S	Y
	AGT	ACC	279 CGA	T TC	ACT	288 TTC	GGC	CCT	297 GGG	ACC	AAA	306 GTG	GAT	ATC	315 AAA	3 '		
	S	-	R CDR3	F		F	G	P	G	T	К	V	D	I	К			

Fig. 14a

LD1-6-17-VH sequence

		9			18			27			36			45		m cc	
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG			AGG		
Q	v	K	L	L	E	S	G	G	G	V	V	Q	P	G	R	S	L
AGA	CTT	63 TCC	TGT	GCA	GCG	TCT	GGA	TTT		TTC	AGT						
 R		s		Α	Α	S	G	F	Т	F	S	S	Y	G GDD1	M	H	W
GTC	CGC	117 CAG	GCT	CCA	GGC	AAG	GGG	135 CTG	GAG	TGG	144 GTG		GAT	153			162 GAT
v	R	Q				K	G	L	E	W	V		D ←			F	D
GGA	GGT	171 AA.T	AAA	CAT	180 TAT	GCA	GAC	TTC	GTG	AAG	198 GGC	CGA		207			216
	- -	N	 К				D	F	٧	K	G	R	F	T	I	S	R
GAC	AAT	225 TCC	AAG		~ ~ .			242	CAA		252 AAC	AGC	СТG 	261 AGA	GTC	GAG	270 GA0
D	 N	 S		N	 T	V	Y	L	Q	М	N	S	L	R	V	E	D
ACG	GCT	279 GTG	TAT	TAC	288 TGT	GCG	AGG	GAT	TAC	TAT	306 AGC	GTT	ACT	315 AAG	AAA	CTC	324 AGA
 T	 A		Y	Y		- А				Y	S	V	T	K	K	L	R
CTC	CAC	333 TAC	TAC	TAC	TAC	ATG	GAC	351 GTC	TGG	GGC	360 AAA	GGG	ACC	369 ACG	GTC	ACC	378 GT0
		Y	Y	Y	Y	M					К						V
			— c	DR3					→								

TCC TCA 3'

Fig. 14b

LD1-6-17-VL sequence

GTG	ATG	9 ACC	CAG	TCT	18 CCA	TCC	TCC	27 CTG	TCT	GCA	36 TCT	GTA	GGA	45 GAC	AGA	GTC	54 ACC
	 M	т	Q	s	P	S	S	L	s	Α	s	V	G	D	R	V	Т
ATC	ACT	63 TGC	CGG	GCA	72 AGT	CAG	GGC	81 ATT	AGA	TAA	90 GAT	TTA	ACC	99 TGG	TAT	CAG	108 CAA
 I	T		 R	λ	s	Q	G	I	R	N	D	L	Т	W	Y	Q	Q
AAA	CCA	117 GGG		GCC	126			125			144		TCC	153 AAT	TTA		162 AG
 K		 G	 К	 A	P	K	L	L	I	Y				И			
GG	GTC	171 CCA	TCA	AGG	180 TTC	AGC	GGC	189 AGT	GGA	TCT	198			CDR2 207 TTC			21
G		P	s	R	F	S	G	s	G	S	G	T	D	F	Т	Ŀ	Т
TA	C AGC	225 AGC	СТG	CAG	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAT	TAC	TGT	261 CTA	CAA	GAT	27 AA
	 S	 S		Q	P	 E	D	F	Α	T	Y	Y	С	L	Q	D	N
AΑ	r TTC	279 : CCG	TAC	ACT	298 TTT	GGC	CAG	297 GGG	ACC	AAG	306 CTG	GAG	ATC	315 AAA			
<u>-</u>	F		Y	 T —→	- 	- - -	Q	G	т	K	L	E	I	K			

Fig. 15a

LD1/2-6-3-VH sequence

CAG (STG	9 AAA	CTG	CTC	18 GAG	TCT	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCG	45 GGG 	GGG	TCC	5 CT
		 K		 L			G				V		P		G	S	
AGA (GTC	63 GCC	TGT	GTA	72 GCG	тст	GGA	81 TTC	ACC	TTC	90 AGG	AAT	TTT	99 GGC 	ATG	CAC	10 TG
·	 V	 А		 V	Α	s	G	F	Т	F	R	N	F	G CDP1	M	H	V
GTC	CGC	117 CAG	GĊT	CCA	126 GGC	AAG	GGG	CTG	GAG	TGG	144 GTG	GCT		153		TTT	16
 V	- <i></i>	 0	 A			- - -					V	Α	F	I	W CDR2	F]
•		-	AAA			GGA	GAC	189 TCC	GTT	AAG	198 GGC	CGA		207			2
	 S		 K	G	Y	G	D	s	V	К	G	R	F	Т	V	S	
GAC	ТАД	225 TCC	AAG			R2 CTC			CAA	ATC	252 AAC	GGC	С Т G	AGA	GCC	GAA	2 . G
		 S				L				М		G	L	R	Α	Ε	
ACG	GCT	279 GTA	TAT	TAT	288 TGT	GCG	AGA	GAG	AAG	GCC	GTT	CGG	GGA	. ATI	AGT	AGA	T -
	 A		 Ү	 Y		 A	R	E		А	v	R	G	Ţ	s	R	_
_						2 C TGG					261	CI)K3 -	369)		
N	 Y	Y	м з —	D	- ∨	 W	G	K	G	 T	T	V	T		S	S	

Fig. 15b

LD1/2-6-3-VL sequence

(STG	ATG	9 ACC	CAG	тст	18 CCA	тсс	TCC	27 CTG	тст	GCA	36 TCT	GTA	GGA	45 GAC	AGA	GTC	54 ACC
	 V	 М	 T	 Q	 S	 Р	- - -	 S	L	s			v	G	D	R	٧	Т
	ATC	ACT	63 TGC	CGG	GCA	72 AGT	CAG	AGC	81 ATT	ATC	AGA	90 TAT	TTA	AAT	99 TGG	TAT		108 CAC
	 I	 T	 C	 R	 A		Q	s		I	R	Y	L	N	W	Y		Н
	AAA	CCA	117 GGG	AAA	GCC	126 CCT			125		CAT	144			153 AGT	TTG	CAA	162 AGT
	 K	 P	 G	К	 A	P	- - -	L	L	I	Н	Т	Α	S	s — CD	L D2	Q	s
	GGG	GTC	171 CCG	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GTA	TCT	198 GGG						216
	 G		P	S	R	F	S	G	S	V	S	G	T	D	F	T	L	T
	ATC	AGC	225 AGT	CTG	CAA	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	270 TAC
		 S		 L	Q										Q		S	
	ACT	ACC	279 CCG	TAC	ACT	288 TTT	GGC	CAG	297 GGG	ACC	AAG	306 CTG	CAG	ATC	315 AAA			
	Ţ	T	P CDR3	Y	_	 F	G	Q	G	Т	К	L	Q	I	K	j		

Fig. 16a

LD1/2-6-33-VH sequence

5'	CAG	GTG	9 AAA	CTG	CTC	18 GAG	TCT	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCG	45 GGG	GGG	TCC	54 CTG
	0	 V	 к	 L	 L	 Е	 S		 G			V	Q	Þ	G	G	s	L
	~	GTC	63 GCC	TGT	GTA	72 GCG	TCT	GGA	81 TTC	ACC	TTC	90 AGG 	TAA	TTT	99 GGC	ATG	CAC	108 TGG
		 V										R	N	F	G	М	H →	W
	GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGG	135 CTG	GAG	TGG	144 GTG			153			162
	 V			 A	 P	 G	- - -	G	L	E	W	V	Α	F	I	W	F	D
	GC A	TOA	171 AAT	AAA	GGA	180 TA T	GGA	GAC	189 TCC	GTT	AAG	198 GGC	CGA		207			216 AGA
				 K	 G	 Y	 G		5	v	K	- G	R	F	Т	V	S	R
					AAC	- CDI	R2 —								261			270 GAC
			 S	 К	 N	 T						N		L	R	Α	Ε	D
	_		279 GTA	LAT	TAT	288 TGT	GCG	, AGA	GAG	, AAG	GCG	GTT	CGG	GGA	315 ATT	AGT	AGA	TAC
		 A					A	 R	E	К	Α	V	R	G	I	S	R	Y
	•			B C ATC	G GAC	342 C GTC	t TGC	G GGC	·			360 ACG	ı	ACC	369 GTC) TC(TCA	\ 3' -
		Y	Y	- <i>-</i>	D	v	W					Т			V	S	S	

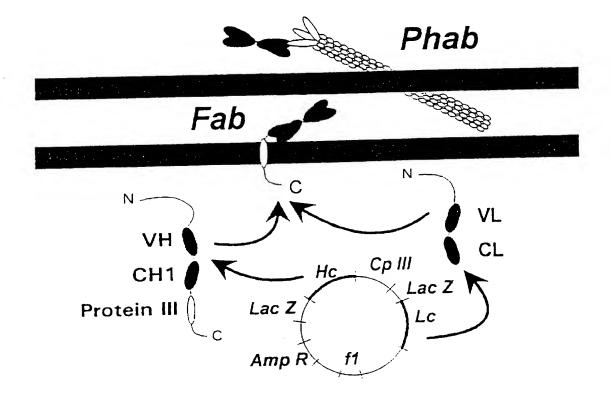
Fig. 16b

LD1/2-6-33-VL sequence

GTG	ATG	9 ACC	CAG	TCT	18 CCA	TCC	TTC	CTG	TCT	GCA	36 TCT	GTA	GGA	45 GAC	AGA	GTC	ACC
	 М	 T	Q	s	P	s	F	L	s	Α	S	V	G	D	R	V	T
ATC	ACT	63 TGC	CGG	GCA	AGT	CAG	AGC	ATT	ATC	AGA	1 P						108 CAC
	 Т	, C		Α	 S	Q	S	I	I	R	Y	L	N	W	Y	Q	Н
AAA	CCA	117 GGG	← AAA	GCC				135 CTG	ATC	CAT	1 A 2		TCC	153 AGT		CAA	
 K	 P	- - - G	 К	- А	- Р	 К	L	L		Н	A	Α	S	s — CD	L	Q	S
			TCA	AGG	180 TTC	AGT	GGC	189 AGT	GTA	TCT	198 GGG	ACA	GAT	207 TTC	ACT	CTC	216
 G	 V	 P	s	 R	 F	-	G	S	V	S	G	Т	D	F	T	L	T
ATC	AGC	225 AGT	CTG	; CAA	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	270
	 S								A					Q	Q		Y
ACT	' ACC	279 : CCG) F TAC	C ACT	288 TTT	GGC	CAG	297 G GGG	, ACC	: AAG	306 CTC	CAG	ATC	315 AAA			
т	T	P		T					Т								

Fig. 17

The pComb3 Expression System



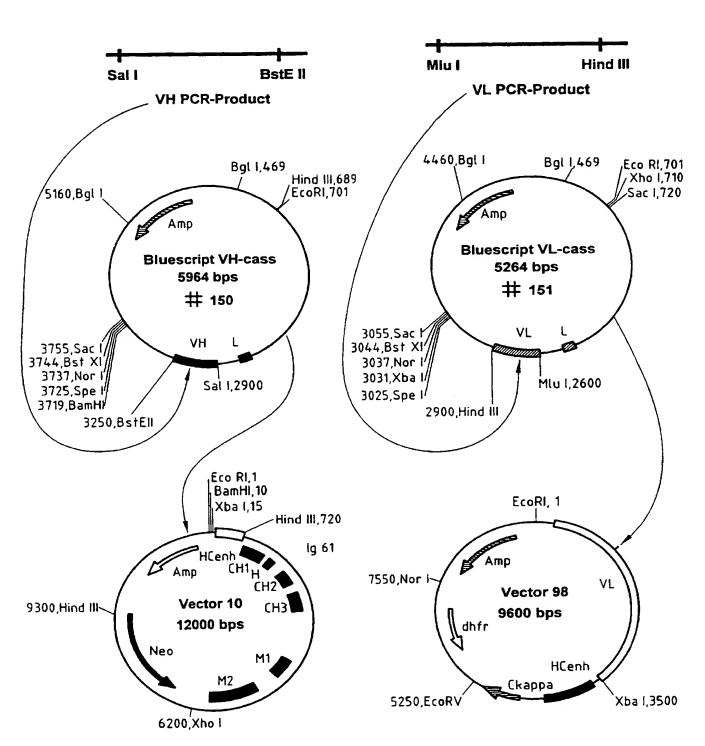


FIG. 18

FIG. 19

INTERNATIONAL SEARCH REPORT

atlanal Application No. PCT/EP 97/03253

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C12N15/13 C12I A61K39/395 G01N33/80 C12N15/62 C07K16/34 According to International Patent Classification (IPC) or to both national classification and IPC **B. FIELDS SEARCHED** Minimum documentation searched (classification system followed by classification symbols) C12N CO7K A61K GO1N IPC 6 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) C. DOCUMENTS CONSIDERED TO BE RELEVANT Relevant to claim No. Citation of document, with indication, where appropriate, of the relevant passages 11-13, Χ SIEGEL D. L. & SILBERSTEIN L. E.: 18-20 "Expression and characterization of recombinant anti-Rh(D) antibodies on filamentous phage: a modelsystem for isolating human red blood cell antibodies by repertoire cloning" BLOOD, vol. 83, no. 8, 15 April 1994, pages 2334-2344, XP000609017 cited in the application see the whole document -/--Further documents are listed in the continuation of box C. Patent family members are listed in annex. X I Special extegories of cited documents : "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the "A" document defining the general state of the art which is not considered to be of particular relevance. invention "E" earlier document but published on or after the international "X" document of particular relevance; the claimed invention filing date cannot be considered novel or cannot be considered to "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention counnet be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or *P* document published prior to the international filing date but "&" document member of the same patent family later than the priority date claimed Date of mailing of the international search report Date of the actual completion of the international search .1 4. 11. 97 3 November 1997 Name and mailing address of the ISA Authorized officer European Patent Office, P.B. 5818 Patentiaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo ni.

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